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(54) Title: MATERIALS AND METHODS FOR TREATMENT OF NEUROLOGICAL DISORDERS INVOLVING OVERACTI-  
VATION OF GLUTAMATERGIC IONOTROPIC RECEPTORS

(57) Abstract: The subject invention pertains to pharmaceutical compositions, articles of manufacture, and methods useful for treatment of neurological conditions related to, or which can be affected by, modulation of glutamate receptor (GluR) activity. The treatment can be either prophylactic in nature or to alleviate symptoms of such neurological conditions. The pharmaceutical compositions of the subject invention include an aromatic amino acid (AAA), an analog or isomer of an AAA, or combinations thereof, and pharmaceutically acceptable carrier or diluent. Methods of the subject invention involve parenterally administering to a patient at least one AAA, an analog or isomer of an AAA, or combinations thereof.

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## DESCRIPTION

### MATERIALS AND METHODS FOR TREATMENT OF NEUROLOGICAL DISORDERS INVOLVING OVERACTIVATION OF GLUTAMATERGIC IONOTROPIC RECEPTORS

#### Cross-Reference to Related Application(s)

This application claims the benefit of patent application Serial No. 09/957,358, filed September 19, 2001, which is hereby incorporated by reference in its entirety, including all figures, tables, and drawings.

#### Background of the Invention

[0001] Glutamate is the principal excitatory neurotransmitter in the mammalian brain and is known to participate in higher order processes, such as development, learning, and memory. As an excitatory amino acid (EAA), glutamate is also involved in neuropathologic events, including cell death, that result from excessive stimulation of post-synaptic neurons (*i.e.*, excitotoxic damage). Glutamate binds or interacts with one or more glutamate receptors (GluRs), which can be differentiated pharmacologically into different classes and subtypes. In the mammalian central nervous system (CNS) there are three main subtypes of ionotropic glutamate receptors (iGluRs), defined pharmacologically by the selective agonists N-methyl-D-aspartate (NMDA), kainite (KA), and  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA).

[0002] The iGluRs are ligand-gated ion channels that, upon binding glutamate, open to allow the selective influx of certain monovalent and divalent cations, thereby depolarizing the cell membrane. In addition, certain iGluRs with relatively high calcium permeability can activate a variety of calcium-dependent intracellular processes.

[0003] During a period of anoxia (*e.g.*, cardiopulmonary resuscitation), ischemic stroke, epileptic seizure, and other types of CNS injury, GluRs of the NMDA, KA, and AMPA subtypes are overactivated (Choi DW [1992] *Neurobiol* 9:1261-96; Zipfel GJ *et al.*, [2000] *J Neurotrauma* 10:857-69; Fountain NB. [2000] *Epilepsia* 41 Suppl 2:S23-30; Tanaka H *et al.* [2000] *Brain Res* 886(1-2):190-207; Pujol R *et al.* [1999] *Ann NY Acad Sci* 884:249-254). The net result of this effect is a massive increase in the concentration of intracellular

calcium, which in turn triggers a deleterious cascade of events leading to neuronal death (Sapolsky RM [2001] *J Neurochem* 76(6):1601-1611). Functional overactivity of iGluRs has also been implicated in a variety of neurodegenerative diseases, such as lateral sclerosis, Alzheimer's disease, Huntington's chorea and AIDS dementia syndrome (Tanaka H *et al.* [2000] *Brain Res* 886(1-2):190-207).

[0004] In the search for safe and efficacious neuroprotective agents, iGluR antagonists remain thought of as promising therapeutic drugs (Gagliardi RJ [2000] *Arq Neuropsiquiatr* 58(2B):583-588). A minimum of 800 neuroprotective trials using GluR antagonists is currently underway worldwide. Among the neuroprotective agents being studied, the most important ones include iGluR antagonists of the NMDA and AMPA subtype, and inhibitors of glutamate release. However, many of these drugs cause significant side effects (*e.g.*, neurotoxicity) that will probably limit their widespread clinical use. For example, the NMDA channel blocker dizocilpine (MK-801) causes neuronal vacuolation in specific areas of the rat brain cortex (Olney JW *et al.* [1989] *Science* 244:1360-1362; Fix AS *et al.* [1994] *Drug Development Research* 32:147-152; Muir KW *et al.* [1995] *Stroke* 26:503-513).

[0005] In contrast to the iGluRs, the metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors capable of activating a variety of intracellular second messenger systems following the binding of glutamate. Activation of mGluRs in mammalian neurons can decrease the activity of ion channels, including ligand-gated channels such as iGluRs. Several subtypes of mGluRs have been isolated by molecular cloning. In addition, the various subtypes of mGluRs have been divided into three groups based on amino acid sequence homologies, the second messenger systems they utilize, and pharmacological characteristics (Nakanishi [1994] *Neuron* 13:1031).

[0006] U.S. Patent No. 6,084,084 (the '084 patent) discloses a human mGluR protein and methods of screening for compounds that bind to the mGluR receptor and modulate its activity, and using such compounds to treat various neurological disorders. The '084 patent also cites several references that teach various mGluR modulators, the majority of which are L-glutamate derivatives.

[0007] A family of substances that have not previously been investigated as modulators of GluR activity are the naturally occurring aromatic amino acids, L-tyrosine, L-tryptophan, and L-phenylalanine. While some phenylalanine derivatives have been investigated as possible agonists or antagonists of mGluR activity, these derivatives showed little or no agonist or antagonist activity on any subtypes of the receptors investigated (Sekiyama N *et al.* [1996] Br J Pharmacol 117:1493-1503).

[0008] Aromatic amino acids have been available as over-the-counter dietary supplements for some time, with their consumption generally viewed as beneficial for their role as biosynthetic precursors for the neurotransmitter precursors serotonin, dopamine, and norepinephrine. In addition, there is research suggesting that oral administration of neurotransmitter precursors may be useful in treatment of some pathologic conditions of the brain, but not others. For example, oral administration of preparations that included tyrosine was found to confer some benefit to patients suffering from multi-infarct dementia and Alzheimer's disease (Meyer JS *et al.* [1977] J Am Geriatr Soc (July) 25(7):289-298), and oral administration of D-phenylalanine conferred some benefit on patients suffering from Parkinson's disease (Heller B *et al.* [1976] Arzheim-Forsch (Drug Res.) 26(4):577-579); however, orally administered tyrosine did not confer a benefit to patients suffering from cocaine dependence (Galloway GP *et al.* [1996] J Psychoactive Drugs (Jul-Sep) 28(3):305-309).

[0009] It is evident that the currently available glutamate receptor modulators may be of limited use, both as research tools and potential therapeutic agents, as a result of their lack of potency and selectivity. Accordingly, there remains a need for safe and efficacious neuroprotective agents.

#### Brief Summary of the Invention

[0010] The subject invention concerns methods for treating a disease or condition which is related to, or which can be affected by, modulation of glutamate receptor (GluR) activity. Particularly, the subject invention concerns methods for treating neurological conditions characterized by excessive activation of glutamatergic ionotropic receptors (iGluR). The treatment can be either prophylactic in nature or to alleviate symptoms of such neurological conditions.

[0011] According to the methods of the subject invention, an AAA is administered in an amount effective to increase the concentration of the AAA within the brain to a level above physiologically normal. For example, the AAA can be administered in an amount effective to bring the patient's AAA blood plasma level within the range of about 200  $\mu\text{M}$  to about 2000  $\mu\text{M}$ . Preferably, the patient's AAA blood plasma level is brought to within the range of about 300  $\mu\text{M}$  to about 1800  $\mu\text{M}$ . More preferably, the patient's AAA plasma level is brought to within the range of about 800  $\mu\text{M}$  to about 1500  $\mu\text{M}$ . However, the appropriate concentration of AAAs in the blood can be adjusted, as permeability of the blood-brain barrier can vary with different disease states. Advantageously, the AAA depresses or inhibits functioning of ionotropic GluRs at excitatory synapses in the brain.

[0012] Neurological conditions characterized by excessive activation of glutamatergic ionotropic receptors (iGluRs) include, but are not limited to, anoxic/hypoxic damage (*e.g.*, cardiopulmonary resuscitation, drowning), traumatic brain injury, spinal cord injury, local anesthetic-induced seizure activity, ischemia (including global ischemia), stroke (including ischemic stroke), ischemic neurodegeneration of the retina, epileptic seizures (including epilepticus), Tourette's syndrome, obsessive-compulsive disorder, drug-induced (*e.g.*, nerve gas-induced) CNS injury, chronic pain syndromes, acute and chronic neurodegenerative disorders (*e.g.*, lateral sclerosis, Alzheimer's disease, Huntington's chorea), AIDS dementia syndrome, cocaine addiction, hypoglycemia, or combinations thereof. In one embodiment, the method of the subject invention comprises parenterally administering to the patient an AAA, an analog or isomer of an AAA, or combinations thereof.

[0013] In a specific embodiment, the neurological condition is selected from the group consisting of ischemia, stroke, traumatic brain injury, hypoglycemia, and epileptic seizure. In another specific embodiment, the neurological condition is not a condition selected from the group consisting of Parkinson's disease, multi-infarct dementia, Alzheimer's disease, and cocaine addiction.

[0014] Unless otherwise indicated, as used herein, the term "AAA" includes naturally occurring aromatic amino acids (*e.g.*, L-tyrosine, L-phenylalanine, and L-tryptophan), their isomers, including optical isomers (*e.g.*, dextrorotatory (D-), levorotatory (L-), or mixtures (DL-) thereof), and analogs thereof. As used herein, the term "analog" is used

interchangeably with the term "derivative". Mixtures of naturally occurring aromatic amino acids, isomers, and analogs, are also contemplated. In a specific embodiment, the optical isomer is not D-phenylalanine.

[0015] The present invention also concerns methods for modulating GluR activity. In one aspect, the method of the subject invention comprises lowering Glu concentration in the synaptic cleft in a patient by administering an AAA. The present invention further pertains to methods for attenuating GluR-mediated miniature excitatory postsynaptic currents (mEPSCs), either during normoxia or ischemic conditions, within a patient by administering an AAA. In addition, the present invention concerns methods for inhibiting GluR activity in a patient by lowering the concentration of Glu in a patient through the administration of an AAA.

[0016] The subject invention also concerns pharmaceutical compositions and articles of manufacture useful in treating a neurological condition characterized by overactivation of an ionotropic glutamatergic receptor. The pharmaceutical compositions and articles of manufacture can include an aromatic amino acid, isomer, or analog thereof, such as 3,5-diiodo-L-tyrosine (DIT) and 3,5-dibromo-L-tyrosine (DBrT).

#### Brief Description of the Drawings

[0017] Figures 1A and 1B show the effect of L-tyrosine, L-phenylalanine, and L-tryptophan on the frequency of miniature excitatory postsynaptic currents (mEPSCs) in rat cultured hippocampal neurons. Examples of mEPSCs, recorded in the presence of the N-methyl-D-aspartate (NMDA) channel blocker MK-801 before, during, and after application of L-tyrosine (L-Tyr), L-phenylalanine (L-Phe), or L-Tryptophan (L-Trp) are shown in Figure 1A. Figure 1B shows summary data for 5-8 experiments demonstrating the effect of L-Tyr, L-Trp, and L-Phe on the frequency of mEPSCs. The frequencies of events were normalized to control values and plotted against the concentration of L-Tyr, L-Trp, and L-Phe. The results are presented as mean $\pm$ S.E.M. \*, P<0.01 compared to control.

[0018] Figures 2A and 2B show the effect of D-tyrosine, D-tryptophan, and D-phenylalanine the frequency of mEPSCs in rat cultured hippocampal neurons. Examples of mEPSCs, recorded in the presence of the NMDA channel blocker MK-801 before, during, and after application of D-phenylalanine (D-Phe), D-tryptophan (D-Trp), and D-tyrosine (D-

Tyr) are shown in Figure 2A. Figure 2B shows summary data for 5-8 experiments demonstrating the effect of D-Tyr, D-Trp, and D-Phe on the frequency of mEPSCs. The frequencies of mEPSCs were normalized to control values and plotted against the concentration of D-Tyr, D-Trp, and D-Phe. The results are presented as means $\pm$ S.E.M. \*,  $P < 0.01$  compared to control.

[0019] Figures 3A-3I show L-Phe depresses the non-NMDA component of GluR-mediated EPSCs in rat hippocampal neurons. Figure 3A shows representative non-N-methyl-D-aspartate-type glutamate receptor (NMDAR)-mediated sEPSC recording from a single neuron before, during (L-Phe, 1 mM), and after application of L-Phe. Horizontal bar denotes L-Phe application. Figures 3B and 3C show summary data for the effect of L-Phe on amplitude and frequency of non-NMDAR-mediated sEPSCs at the same conditions. Data was normalized to control values and plotted as mean $\pm$ SEM. Figure 3D shows representative non-NMDAR-mediated mEPSCs recordings from a single neuron at the same conditions. Figures 3E and 3F show cumulative probability distribution of the mEPSC inter-event interval and amplitude. Summary data is expressed as mean $\pm$ SEM of 39 cells. Error bars have been removed for clarity. Figures 3G and 3H show summary data for the effect of L-Phe on frequency and amplitude of non-NMDAR-mediated mEPSCs. Figure 3I shows concentration-response relationship of L-Phe-induced attenuation of non-NMDAR-mediated mEPSC frequency. Data was normalized to control values and plotted against the concentration of L-Phe. The equation used for fitting the data and the curve fitting parameters are shown in the figure. Data is expressed as mean $\pm$ SEM of 5 to 35 cells. For all panels: Intervention vs. Control: \*,  $P < 0.01$ .

[0020] Figures 4A-4D show that 3,5-Diiodo-L-tyrosine(DIT) and 3,5-dibromo-L-tyrosine (DBrT) depress the non-NMDA component of GluR-mediated EPSCs in rat hippocampal neurons. Figure 4A shows representative non-NMDAR-mediated sEPSC recording from a single neuron before, during (DIT, 1 mM), and after application of L-Phe. Figure 4B shows representative non-NMDAR-mediated single sEPSCs recordings from a single neuron at the same conditions. Figure 4C summary data for the effect of L-Phe on amplitude of non-NMDAR-mediated sEPSCs. Data is expressed as mean $\pm$ SEM of 9 cells. Figure 4D concentration-response relationships of DIT- and DBrT-induced attenuation of non-NMDAR-mediated mEPSC frequency. Data was normalized to control values and

plotted against the concentration of L-Phe. The equation used for fitting the data and the curve fitting parameters are shown in the figure. Data is expressed as mean $\pm$ SEM of 4-6 cells. For all panels: Intervention vs. Control: \*,  $P < 0.05$ . Dotted line represents concentration-response relationship of L-Phe-induced attenuation of non-NMDAR-mediated mEPSC frequency (from Figure 3I).

[0021] Figures 5A-5H show that the effect of L-Phe on mEPSCs in rat hippocampal neurons is specific. Figure 5A shows that L-Phe changes neither frequency nor amplitude of GABAR-mediated mIPSCs. Representative GABAR-mediated mIPSCs (Figure 5A) and averaged mIPSCs (Figure 5B) recordings from the same neuron before (control, 1), during (L-Phe, 1 mM; 2), and after (wash, 3) application of L-Phe. Cumulative probability distribution of the mIPSC inter-event interval (Figure 5C), and amplitude (Figure 5D) at conditions 1 and 2 ( $n=11$ ). Figure 5B shows that the neutral amino acid L-leucine (L-Leu) attenuates neither frequency nor amplitude of non-NMDAR-mediated mEPSCs. Representative non-NMDAR-mediated mEPSCs (Figure 5E) and averaged mEPSCs (Figure 5F) recordings from the same neuron before (control, 1), during (L-Leu, 1 mM; 2), and after (wash, 3) application of L-Leu. Cumulative probability distribution of the mEPSC inter-event interval (Figure 5G), and amplitude (Figure 5H) at conditions 1 and 2 ( $n=4$ ). Error bars have been removed for clarity.

[0022] Figures 6A-6H shows that the inhibitory effect of L-Phe on glutamatergic spontaneous EPSCs in rat hippocampal neurons depends on the concentration of glycine. Representative GluR-mediated (Figures 6A and 6B) and NMDAR-mediated (Figures 6C and 6D) spontaneous EPSCs before, during (L-Phe, 1 mM) and after application of L-Phe are depicted in the upper panels. Histograms summarizing the effect of L-Phe on amplitude and frequency of sEPSCs are depicted in the bottom panels. Horizontal bars denote L-Phe application. Concentrations of glycine (Gly, 0.1 and 10  $\mu$ M) in extracellular solution are shown in each figure. All measurements were performed at a holding membrane potential of  $-30$  mV and in the presence of  $Mg^{2+}$  (1 mM). Each representative recording was made from a single neuron. Summary data is expressed as mean $\pm$ SEM of 8-9 cells. Intervention vs. Control:  $\S$ ,  $P < 0.05$ ; \*,  $P < 0.01$ .

[0023] Figures 7A-7G show that L-Phe differentially affects non-NMDAR- and NMDAR-mediated currents in rat hippocampal neurons. Figure 7A shows the representative



recordings of the non-NMDA component of current activated by exogenous glutamate in the absence (1) and presence (2) of L-Phe (1mM). Horizontal bars denote glutamate (3000  $\mu$ M and 3  $\mu$ M) application. All recordings were made from a single neuron. Figure 7B shows the summary data for 8 experiments as shown in Figure 7A. Peak  $I_{Glu}$  in the presence of L-Phe was normalized to control values and plotted as mean  $\pm$  SEM. Figures 7C and 7D show that L-Phe did not affect the NMDA-activated current ( $I_{NMDA}$ ) recorded in the presence of a high concentration of glycine. Figure 7C show representative traces of  $I_{NMDA}$  recorded from a single neuron at 10  $\mu$ M (left, gray bar) and 0.1  $\mu$ M (right, black bar) of glycine in the absence (1) and presence (2) of L-Phe (1 mM). Horizontal bars denote NMDA (3  $\mu$ M) application. Figure 7D show the summary data for 8 experiments as shown in Figure 7C. Peak  $I_{NMDA}$  in the presence of L-Phe was normalized to control values and plotted as mean  $\pm$  SEM. Figures 7E-7G show that L-Phe significantly decreased frequency but not amplitude of NMDAR-mediated mEPSCs recorded in the presence of glycine (10  $\mu$ M). Figure 7E show the averaged NMDAR-mediated mEPSCs recorded from the same neuron before (control), during (L-Phe, 1 mM), and after (wash) application of L-Phe. Figures 7F and 7G show bars that represent mean  $\pm$  SEM of 20 cells for the effect of L-Phe on the amplitude (Figure 7F) and the frequency (Figure 7G) of NMDAR-mediated mEPSCs. For all panels: Intervention vs. Control: \*,  $P < 0.05$ .

[0024] Figures 8A-8F show that L-Phe decreases both frequency and amplitude of non-NMDAR-mediated mEPSCs in rat cerebrocortical neurons. Non-NMDAR-mediated mEPSCs were recorded from neurons on days 11-14 in the culture at conditions similar to those applied for hippocampal neurons. Holding membrane potential was  $-70$  mV. Figure 8A shows representative non-NMDAR-mediated mEPSCs recordings from a single neuron before (control), during (L-Phe, 1 mM), and after (wash) application of L-Phe. Figures 8B and 8C show cumulative probability distribution of mEPSC inter-event interval and amplitude. Summary data is provided from 8 cells. Error bars have been removed for clarity. Figure 8D shows the averaged mEPSCs recorded from the same neuron shown in panel A. Figures 8E and 8F show the summary data for the effect of L-Phe on frequency and amplitude of non-NMDAR-mediated mEPSCs. Bars represent mean  $\pm$  SEM of 8 cells (\*,  $P < 0.01$ , intervention vs. control).

[0025] Figures 9A-9F show that L-Phe depresses non-NMDAR-mediated mEPSCs in mouse hippocampal neurons. Figures 9A and 9D show representative original and averaged non-NMDAR-mediated mEPSCs recorded from a single neuron before (control, 1), during (L-Phe, 1 mM; 2), and after (wash, 3) application of L-Phe. Figures 9B and 9C show the cumulative probability distribution of mEPSC inter-event interval and amplitude at conditions 1 and 2 (n=9). Error bars have been removed for clarity. Figures 9E and 9F show the summary data for the effect of L-Phe on frequency and amplitude of non-NMDAR-mediated mEPSCs. Bars represent mean  $\pm$  SEM of 9 cells for frequency (panel C, \*,  $P < 0.01$ , intervention vs. control) and amplitude (panel E; \*,  $P < 0.001$ , intervention vs. control).

[0026] Figures 10A-10C show the effect of L-Phe on mEPSCs in rat cultured hippocampal neurons during energy deprivation (ED). To induce ED, glucose was replaced with 2-deoxyglucose, and sodium cyanide (NaCN) was added. Examples of mEPSCs, recorded in the presence of the NMDA channel blocker MK-801 in control, during ED, during ED in the presence of L-Phe, and during ED after washout of L-Phe are shown in Figure 10A. Figure 10B shows an example of averaged mEPSCs recorded from the same neuron shown in Figure 10A. Figure 10C shows summary data for four experiments demonstrating the effect of L-Phe on the frequency of mEPSCs. The frequency of mEPSCs during ED was taken as 100%. \*,  $P < 0.01$  compared to the values of frequency of mEPSCs during ED only.

[0027] Figures 11A and 11B show that treatment with AAAs markedly decreased cell death caused by oxygen glucose deprivation (Hypoxia/Ischemia Protocol, H/I). Neuronal cultures prepared from the cerebral cortices of newborn rats were grown in normal DMEM containing high levels of aromatic amino acids (group 1) or DMEM containing only 50  $\mu$ M each of tyrosine, tryptophane and phenylalanine (group 2), for 10-12 days. Cultures were then exposed to H/I conditions as follows: Media were removed from each dish and replaced with 1ml of the corresponding DMEM without glucose. Cells were immediately placed in a Billups-Rothenberg chamber and exposed to hypoxic conditions for 15 minutes. After this, the glucose free media were removed and replaced with the original media. One set of cultures from each group was used immediately to assess the levels of LDH in the media (to evaluate necrotic cell death, Figure 11A), and for analysis of cleaved caspase-3 (to evaluate apoptotic cell death, Figure 11B) by Western Blot (time 0 in the data). The remaining cells

from each group were returned to the CO<sub>2</sub> incubator, and LDH/caspase-3 analyses performed at 5, 9, 12, 18, 24 and 48hr. LDH and cleaved caspase-3 analyses were also performed on cultures from groups 1 and 2 that were not exposed to H/I conditions. These "controls" are plotted on the y-axis (LDH), or are listed as Con in the Western Blot.

[0028] Figures 12A and 12B show that treatment with AAAs&Ds markedly decreased infarct volume in rat brain caused by middle cerebral artery occlusion. Figure 12A shows that triphenyltetrazolium chloride (TTC)-stained coronal sections of brain from a representative rat receiving 0.9% NaCl (Figure 12A, top) or DIT (Figure 12A, bottom). Note the decreased infarct size in DIT-treated rat. Figure 12B shows infarct volumes at 48 hours after reperfusion expressed as % of the contralateral hemisphere.

[0029] Figures 13–15 show formulas representing AAA analogs of the subject invention.

#### Detailed Disclosure of the Invention

[0030] The subject invention concerns methods for treating a neurological condition which is related to, or which can be affected by, modulation of glutamate receptor (GluR) activity. The treatment can be either prophylactic in nature or to alleviate symptoms of such neurological conditions.

[0031] Particularly, the subject invention concerns methods for treating neurological conditions characterized by excessive activation of glutamatergic ionotropic receptors (iGluR). Neurological conditions characterized by excessive activation of glutamatergic ionotropic receptors (iGluRs) include, but are not limited to, anoxic/hypoxic damage (*e.g.*, cardiopulmonary resuscitation, drowning), traumatic brain injury, spinal cord injury, local anesthetic-induced seizure activity, ischemia (including global ischemia), stroke (including ischemic stroke), ischemic neurodegeneration of the retina, epileptic seizures (including epilepticus), Tourette's syndrome, obsessive-compulsive disorder, drug-induced (*e.g.*, nerve gas-induced) CNS injury, chronic pain syndromes, acute and chronic neurodegenerative disorders (*e.g.*, lateral sclerosis, Alzheimer's disease, Huntington's chorea), AIDS dementia syndrome, cocaine addiction, hypoglycemia, or combinations thereof. In a specific embodiment, the neurological condition is selected from the group consisting of ischemia, stroke, traumatic brain injury, hypoglycemia, and epileptic seizure. In another specific

embodiment, the neurological condition is not a condition selected from the group consisting of Parkinson's disease, multi-infarct dementia, Alzheimer's disease, and cocaine addiction. In one embodiment, the method of the subject invention comprises parenterally administering one or more AAAs to a patient.

[0032] Unless otherwise indicated, as used herein, the term "AAA" includes naturally occurring aromatic amino acids (*e.g.*, L-tyrosine, L-phenylalanine, and L-tryptophan), their isomers, including optical isomers (*e.g.*, dextrorotatory (D-), levorotatory (L-), or mixtures thereof (DL-)), and analogs thereof. Mixtures of naturally occurring aromatic amino acids, isomers, and analogs are also contemplated. Examples of mixtures of the naturally occurring aromatic amino acids include, but are not limited to L-tyrosine and L-tryptophan; L-tyrosine and L-phenylalanine; L-tryptophan and L-phenylalanine; and L-tyrosine, L-tryptophan, and L-phenylalanine. Each of the naturally occurring amino acids in these mixtures can be substituted with an isomer or analog. In a specific embodiment, the optical isomer is not D-phenylalanine.

[0033] In a preferred embodiment, the subject invention involves treating a patient suffering from a neurological condition characterized by excessive activation of iGluRs. Also in a preferred embodiment, the AAA has an effect on a physiological or pathophysiological activity. By way of illustration, and not limitation, these activities can include convulsions, neuroprotection, neuronal death, neuronal development, central control of cardiac activity, waking, motor control, and control of vestibulo ocular reflex. In another embodiment, the method of the subject invention further comprises diagnosis and/or monitoring of a neurological condition within the patient, wherein the condition is characterized by excessive activation of iGluRs. Advantageously, the AAA depresses or inhibits functioning of ionotropic GluRs at excitatory synapses in the brain.

[0034] The subject invention is at least partly based on the observation that aromatic amino acids (AAAs) (L- and D- forms of phenylalanine, tyrosine, and tryptophan) diminish iGluR-mediated mEPSCs in cultured rat hippocampal neurons. The AAAs significantly diminished control mEPSCs during normoxia and significantly diminished mEPSCs which were augmented during ischemic insult (energy deprivation). To induce energy deprivation, glucose was replaced with 2 mM 2-deoxyglucose, and 5mM sodium cyanide (NaCN) was added. Without being limited by theory, the experimental results suggest that the inhibition

of GluR-mediated synaptic transmission occurs due to a decrease of Glu concentration in the synaptic cleft. AAAs may decrease concentration of Glu by one or more of the following: (1) activation of receptors which inhibit Glu release presynaptically, such as metabotropic GluRs (mGluRs), M<sub>2</sub> muscarinic,  $\alpha_2$ -adrenergic and  $\gamma$ -aminobutyric acid subtype B (GABA<sub>B</sub>) receptors; (2) inhibition of the vesicular release machinery of Glu; or (3) stimulation of Glu uptake.

[0035] Using the patch clamp technique, the present inventors have found that in rat and mouse hippocampal and cerebrocortical cultured neurons, L-Phe and other aromatic amino acids [L- and D-forms of tyrosine (Tyr) and tryptophan (Trp)], significantly and reversibly depressed basal and augmented (via energy deprivation) GluR-mediated excitatory postsynaptic currents (EPSCs) in a concentration dependent manner (50% inhibition at  $0.98 \pm 0.13$  mM). Furthermore, analogs of L-Tyr, 3,5-diiodo-L-tyrosine (DIT) and 3,5-dibromo-L-tyrosine (DBrT), were found to be a 10 fold more potent inhibitor of EPSCs than their precursors, as described in the examples and shown in the Figures.

[0036] The depressant effect of aromatic amino acids and their analogs on glutamatergic synaptic transmission was specific. Thus, L-leucine, an amino acid that competes with L-Phe for brain transporter, had no effect on mEPSCs. Unlike GluR s/mEPSC, GABA receptor-mediated inhibitory postsynaptic currents (IPSCs) were not attenuated by AAAs.

[0037] Three distinct mechanisms mediate the effect of AAAs on glutamatergic synaptic transmission: 1) attenuation of glutamate release from the presynaptic neuron; 2) competition for the glycine-binding site of NMDARs; and 3) competition for the glutamate-binding site of non-NMDAR subtypes. Glycine-independent attenuation of NMDAR s/mEPSC frequency suggest decreased presynaptic glutamate release caused by AAAs, whereas decreased amplitudes of NMDAR and non-NMDAR s/mEPSCs are consistent with competition of AAAs for the glycine- and glutamate-binding sites of NMDARs and non-NMDARs, respectively.

[0038] Consistent with an antiglutamatergic action, AAAs significantly decreased oxygen glucose deprivation-induced injury in rat hippocampal cultured neurons, as described in the Examples. In addition, treatment with AAAs significantly decreased (by 70%) infarct

volume in rat brain caused by middle cerebral artery occlusion. These findings indicate the potential use of aromatic amino acids, their isomers and their analogs, as safe and effective therapeutic agents to treat neurological disorders that involve excessive activation of GluRs.

[0039] In accordance with the subject invention, an AAA can be administered to a patient through a variety of parenteral routes. For example, an AAA can be administered to the patient intravenously or nasally. Alternatively, an AAA can be administered directly into the patient's brain, through microdialysis techniques, for example.

[0040] AAA analogs of the subject invention include analogs of naturally occurring AAAs and analogs of their isomers. Analogs of the subject invention can be substituted at various positions. Figures 13-15 show formulas representing AAA analogs of the subject invention. It should be understood that while the AAA analogs of the subject invention can be produced by modifying the naturally occurring aromatic amino acids (phenylalanine, tryptophan, and tyrosine), it is contemplated that other starting materials (*e.g.*, other amino acids) can be utilized to produce the AAA analogs of the subject invention, using methods of organic synthesis known to those skilled in the art. As used herein, the terms "analog" and "derivative" are used interchangeably.

[0041] Referring now to each of the formulas in Figures 13 through 15,  $R^1$  and  $R^2$ , which may be the same or different, can be H, alkyl, alkenyl, alkynyl, halogen, or alkoxy.  $R^3$  can be H, O, alkyl, alkenyl, alkynyl, halogen, or alkoxy.  $R^4$  can be H, alkyl, alkenyl, alkynyl, halogen, or alkoxy, but is not present when  $R^3$  is O.  $R^5$  can be H, alkyl, alkenyl, alkynyl, halogen, or alkoxy.

[0042] In one embodiment, in the formulas shown in Figure 13 and Figure 15, the pair of substituents,  $R^3$  and  $R^4$ , can together form a cyclic group, wherein the resulting ring structure is selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl. The resulting ring structure can optionally be benzofused at any available position.

[0043] As used in the specification, the term "alkyl" refers to a straight or branched chain alkyl moiety. In one embodiment, the alkyl moiety is  $C_{1-8}$  alkyl, which refers to an alkyl moiety having from one to eight carbon atoms, including for example, methyl, ethyl,

propyl, isopropyl, butyl, *tert*-butyl, pentyl, hexyl, octyl, and the like. In another embodiment, the alkyl moiety is C<sub>1-3</sub> alkyl.

[0044] The term "alkenyl" refers to a straight or branched chain alkyl moiety having in addition one or more carbon-carbon double bonds, of either E or Z stereochemistry where applicable. In one embodiment, the alkenyl moiety is C<sub>2-6</sub> alkenyl, which refers to an alkenyl moiety having two to six carbon atoms. This term would include, for example, vinyl, 1-propenyl, 1- and 2- butenyl, 2- methyl-2-propenyl, and the like.

[0045] The term "alkynyl" refers to a straight or branched chain alkyl moiety having in addition one or more carbon-carbon triple bonds. In one embodiment, the alkynyl moiety is C<sub>2-6</sub> alkynyl, which refers to an alkynyl moiety having two to six carbon atoms. This term would include, for example, ethynyl, 1-propynyl, 1- and 2- butynyl, 1- methyl-2-butyne, and the like.

[0046] The term "alkoxy" refers to an alkyl-O- group, in which the alkyl group is as previously described.

[0047] The term "halogen" refers to fluorine, chlorine, bromine, or iodine.

[0048] The term "cycloalkenyl" refers to an alicyclic moiety having from three to six carbon atoms and having in addition one double bond. This term includes, for example, cyclopentenyl and cyclohexenyl.

[0049] The term "heterocycloalkyl" refers to a saturated heterocyclic moiety having from two to six carbon atoms and one or more heteroatom from the group N, O, S (or oxidized versions thereof) which may be optionally benzofused at any available position. This includes for example azetidyl, pyrrolidinyl, tetrahydrofuranyl, piperidinyl, benzodioxole and the like.

[0050] The term "heterocycloalkenyl" refers to an alicyclic moiety having from three to six carbon atoms and one or more heteroatoms from the group N, O, S and having in addition one double bond. This term includes, for example, dihydropyran.

[0051] The term "aryl" refers to an aromatic carbocyclic ring, optionally substituted with, or fused with, an aryl group. This term includes, for example phenyl or naphthyl.

[0052] The term "heteroaryl" refers to aromatic ring systems of five to ten atoms of which at least one atom is selected from O, N, and S, and optionally substituted with an aryl group substituent. This term includes for example furanyl, thiophenyl, pyridyl, indolyl, quinolyl and the like.

[0053] The term "aryl group substituent" refers to a substituent chosen from halogen, CN, CF<sub>3</sub>, CH<sub>2</sub>F, and NO<sub>2</sub>.

[0054] The term "benzofused" refers to the addition of a ring system sharing a common bond with the benzene ring.

[0055] The term "cycloimidyl" refers to a saturated ring of five to ten atoms containing the atom sequence  $-C(=O)NC(=O)-$ . The ring may be optionally benzofused at any available position. Examples include succinimidoyl, phthalimidoyl and hydantoinyl.

[0056] The term "optionally substituted" means optionally substituted with one or more of the groups specified, at any available position or positions.

[0057] It will be appreciated that the AAA analogs according to the invention can contain one or more asymmetrically substituted carbon atoms (*i.e.*, chiral centers). The presence of one or more of these asymmetric centers in an analog of the formulas shown in Figures 13-15 can give rise to stereoisomers, and in each case the invention is to be understood to extend to all such stereoisomers, including enantiomers and diastereomers, and mixtures including racemic mixtures thereof.

[0058] Isomers and analogs can be used according to the subject invention so long as the isomers or analogs exhibit the desired biological activity. Biological activity characteristics can be evaluated, for example, through the use of binding assays, or assays that measure cellular response.

[0059] An isomer or analog having the capability to modulate ionotropic glutamate receptor (iGluR) activity and/or metabotropic glutamate receptor (mGluR) activity would be considered to have the desired biological activity in accordance with the subject invention. For therapeutic applications, an isomer or analog of the subject invention preferably has the



capability to inhibit ionotropic glutamate receptor (iGluR) activity and to modulate metabotropic glutamate receptor (mGluR) activity.

[0060] The subject invention brings new, safe, and highly effective treatments to the field of neuroprotective drugs. Specifically, naturally occurring aromatic amino acids (AAAs), including L-Tyrosine (L-Tyr), L-Phenylalanine (L-Phe), and L-Tryptophan (L-Trp), significantly and reversibly attenuate GluR-mediated miniature excitatory postsynaptic currents (mEPSCs) in hippocampal neurons. As demonstrated by the Examples described herein, the AAAs diminish control mEPSCs during normoxia and diminish mEPSCs which are augmented during ischemic insult (energy deprivation, ED).

[0061] The AAAs useful according to the subject invention serve as safe, highly effective, and reversible pharmacological tools to acutely and/or chronically treat many neurological disorders that involve excessive activation of iGluRs. The AAAs effectively inhibit GluR-mediated mEPSCs during normoxia, as well as GluR-mediated mEPSCs, which are augmented during ischemic conditions (energy deprivation, ED). This effect of AAAs on GluR-mediated mEPSCs in cultured hippocampal neurons represents a cellular surrogate of the neuroprotective action of AAAs in a variety of neurological disorders.

[0062] According to the methods of the subject invention, an AAA is administered in an amount effective to increase the concentration of the AAA within the brain to a level above physiologically normal. For example, an AAA can be administered in an amount sufficient to bring the patient's AAA blood plasma level within the range of about 200  $\mu\text{M}$  to about 2000  $\mu\text{M}$ . Preferably, the patient's AAA blood plasma level is brought to within the range of about 300  $\mu\text{M}$  to about 1800  $\mu\text{M}$ . More preferably, the patient's AAA blood plasma level is brought to within the range of about 800  $\mu\text{M}$  to about 1500  $\mu\text{M}$ . However, the appropriate concentration of AAAs in the blood for neuroprotection can be adjusted, as the permeability of the blood-brain barrier can vary markedly with different disease states. In addition, the precise dosage will depend on a number of clinical factors, for example, the type of patient (e.g., human, non-human mammal, or other animal), age of the patient, and the condition under treatment and its severity. A person having ordinary skill in the art would readily be able to determine, without undue experimentation, the appropriate dosages required to achieve the appropriate levels.

[0063] In another embodiment, the methods of the subject invention comprise co-administering a facilitating substance that can enhance uptake of the AAA across the blood-brain barrier, thereby more efficiently raising the concentration of the AAA within the brain, and/or increases the activity of the AAA that is already present in the brain (*e.g.*, endogenously or exogenously present). As used herein, the term "co-administering" means including the facilitating substance within a composition that also comprises the AAA, or separately administering the facilitating substance before, during, or after administration of the AAA. Examples of facilitating substances include, but are not limited to, agents that enhance AAA transport, enhance maximum activity or affinity for the AAA, and/or agents that promote binding of the AAA to receptors in neuronal tissue (*e.g.*, allosteric enhancer).

[0064] As used herein, the terms "treating" or "treatment" refer to prevention, reduction, or alleviation of at least one symptom associated with the particular neurological disorder.

[0065] A "patient" refers to a human, non-human mammal, or other animal in which modulation of an ionotropic glutamate receptor (iGluR) will have a beneficial effect. Patients in need of treatment involving modulation of iGluRs can be identified using standard techniques known to those in the medical profession.

[0066] Mammalian species which benefit from the disclosed methods of treatment include, and are not limited to, humans, non-human primates (*e.g.*, apes, chimpanzees, orangutans, monkeys); domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, hamsters, Vietnamese pot-bellied pigs, rabbits, and ferrets; domesticated farm animals such as bovines, buffalo, bison, horses, donkey, swine, sheep, and goats; exotic animals typically found in zoos, such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros, giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, opossums, raccoons, pandas, hyena, seals, sea lions, elephant seals, otters, porpoises, dolphins, and whales.

[0067] The AAAs utilized in the subject invention can be obtained through a variety of methods known in the art. For example, the naturally occurring levorotatory forms of tyrosine, tryptophan, and phenylalanine can be produced by recombinant technology, as

disclosed in U.S. Patent No. 5,605,818. Methods for artificial synthesis of amino acids, isomers, and analogs are also known to those skilled in the art.

[0068] The AAAs utilized in the subject invention can be independent residues, or residues linked with other residues, which are either the same or different (e.g., a fusion protein or fusion peptide). For example, two or more residues can be linked by peptide bonds (forming a peptide), or hydrocarbon linkages appropriate for the AAAs. There may be, for example, two or more residues connected through at least one residue that is cleaved by an enzyme (e.g., a proteolytic enzyme). The enzyme can be endogenous to the patient, or exogenous to the patient and co-administered.

[0069] A further aspect of the present invention provides a method of modulating the activity of a glutamate receptor (e.g., a metabotropic or ionotropic glutamate receptor), and includes the step of contacting the receptor with an AAA that modulates one or more activities of the glutamate receptor, in general, either stimulating activity or inhibiting activity of the receptor. The method can be carried out *in vivo* or *in vitro*. The contacting step can be carried out with the receptor at various levels of isolation. For example, the AAA can be placed in contact with the receptor while the receptor is associated with tissue, the cell (e.g. neurons or glia), or fully isolated. The subject invention also provides methods for inhibiting GluR-mediated synaptic transmission between neurons and/or decreasing Glu concentration in the synaptic cleft by the administration of an AAA. These methods can be carried out *in vivo* or *in vitro*.

[0070] High blood concentrations of L-Phe (>1200  $\mu$ M versus 55-60  $\mu$ M in healthy patients) cause the neurological disease phenylketonuria (PKU) (Knox WE [1972] Stanbury JB *et al.*, eds., 3<sup>rd</sup> ed., McGraw Hill, New York, pp. 266-295; Scriver CR *et al.* [1989] Scriver *et al.*, eds., McGraw-Hill, New York, pp. 495-546). Unless diagnosed and treated early in life with a L-Phe-restricted diet, irreversible brain damage occurs (Berry HK *et al.* [1979] Dev Med Child Neurol 21:311-320; Pennington BF *et al.* [1985] Am J Ment Defic 89:467-474). However, high concentrations of L-Phe are harmful only during the first years of life, and only during chronic exposure to elevated concentrations of this amino acid. Phenylketonuric patients typically discontinue their therapeutic special diet when they reach adulthood. All PKU-related studies converge on the same conclusion that after the age of 10 years, IQ development is stable for different degrees of dietary relaxation (Burgard P [2000]

Eur J Pediatr 159 (Suppl 2): S74-S79). Thus, in one embodiment, the patient administered the AAA is greater than about ten years old.

[0071] While the AAA can be administered as an isolated compound, it is preferred to administer the AAA in the form of a pharmaceutical composition. The subject invention thus further provides pharmaceutical compositions comprising an AAA as an active ingredient, or physiologically acceptable salt(s) thereof, in association with at least one pharmaceutically acceptable carrier or diluent. The pharmaceutical composition can be adapted for various forms of parenteral administration, such as intravenous and nasal routes. Administration can be continuous or at distinct intervals as can be determined by a person skilled in the art. In another aspect, the subject invention concerns novel derivatives of L-tyrosine, including 3,5-diiodo-L-tyrosine (DIT) and 3,5-dibromo-L-tyrosine (DBrT), which are useful as AAA analogs.

[0072] The pharmaceutical compounds of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* (Martin EW [1995] Easton Pennsylvania, Mack Publishing Company, 19<sup>th</sup> ed.) describes formulations which can be used in connection with the subject invention. Formulations suitable for parenteral administration include, for example, aqueous sterile injection solutions, which may contain antioxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, etc. It should be understood that in addition to the ingredients particularly mentioned above, the formulations of the subject invention can include other agents conventional in the art having regard to the type of formulation in question.

[0073] The subject invention also provides an article of manufacture useful in treating a neurological condition characterized by overactivation of an ionotropic glutamatergic

receptor. The article contains a pharmaceutical composition containing an AAA, and a pharmaceutically acceptable carrier or diluent. The article of manufacture can be, for example, an intravenous bag, a syringe, a nasal applicator, or a microdialysis probe. The article of manufacture can also include printed material disclosing instructions for the parenteral treatment of the neurological condition. The printed material can be embossed or imprinted on the article of manufacture and indicate the amount or concentration of the AAA, recommended doses for parenteral treatment of the neurological condition, or recommended weights of individuals to be treated.

[0074] As used herein, the terms "combinations" and "mixtures" are used interchangeably to include subcombinations or submixtures.

[0075] The terms "comprising", "consisting of", and "consisting essentially of" are defined according to their standard meaning and may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

[0076] All patents, patent applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

#### Materials and Methods

[0077] Cell preparation. Hippocampi or cerebrocortex were dissected from newborn rats or mice, and treated with 0.25% trypsin to dissociate cells using procedures previously described (Chandler, L.J. *et al.*, [1997] *Mol. Pharmacol.*, 51:733-40). Dissociated cells were resuspended in Neurobasal Medium containing B-27 serum-free supplement (Invitrogen Life Technologies, Carlsbad, CA), and were plated in poly-L-lysine coated 35 mm Nunc plastic tissue culture dishes ( $1.5 \times 10^6$  cells/dish/2ml media). Cells were cultured in an atmosphere of 5% CO<sub>2</sub>/95% air, and 50% of the media was replaced every 3 days. Neurons were used for electrophysiological recordings 12 to 27 days after plating.

[0078] Electrophysiological recordings. Electrophysiological recordings of spontaneous and miniature EPSCs in cultured hippocampal and cerebrocortical neurons were made in the whole cell configuration of the patch-clamp technique (Hamill, O.P. *et al.*, [1981] *Pflugers Arch.*, 391:85-1000) using an Axopatch 200B amplifier (Axon Instruments,

Foster City, CA). Patch microelectrodes were pulled from 1.5 mm borosilicate glass tubing using a two-stage vertical pipette puller (Narishige, East Meadow, NY). When filled with recording solution, patch microelectrodes had a resistance of 3-5 M $\Omega$ . For rapid application of agonist-containing solutions to neurons, the SF-77B system (Warner Instrument Corp., Hamden, CT) was used. Current data was digitized on-line using a DigiData 1200A analog-to-digital board and stored on the hard disc of an IBM compatible Pentium computer (GP7-600 MHz, Gateway Computer, Sioux City, ND). Voltage-clamp experimental protocols were performed using the software program pCLAMP7 (Axon Instruments). The experiments were performed at room temperature (22-23 °C). Spontaneous EPSCs were recorded in the presence of 1 mM Mg<sup>2+</sup> in TTX-free extracellular solution at a holding potential of -30 mV. To isolate miniature EPSCs, action potential propagation was blocked by TTX. Strychnine (1  $\mu$ M) and bicuculline (20  $\mu$ M) or picrotoxin (100  $\mu$ M) were added to the extracellular solution to block glycine and GABA receptors, respectively. In order to study the effect of L-Phe on GABAR-mediated mIPSCs, bicuculline (20  $\mu$ M) in the extracellular solution and Cs gluconate (135 mM) in the intrapipette solution were replaced with NBQX (5  $\mu$ M) and with KCl (135 mM), respectively. All mEPSCs and IPSCs were recorded at a holding potential of -60 mV. The digitized data were analyzed off-line using the Mini-Analysis Program (Synaptosoft, Leonia, NJ) with a detection threshold for sEPSC and mEPSC amplitude set at  $\geq 50$  pA and  $\geq 8$  pA, respectively. Miniature EPSCs were identified and confirmed by analyzing the rise time decay time, and waveform of individual spontaneous events.

[0079] Solution and drugs. The extracellular control solution contained (in mM): NaCl 140, KCl 4, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 1, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 10, and glucose 11. The pH of the extracellular solution was adjusted to 7.4 using NaOH. Tetrodotoxin (TTX, 0.3-1  $\mu$ M) was added to the external solution to block voltage-gated Na<sup>+</sup> channels. The solution for filling the patch electrodes contained (in mM): Cs gluconate 135, NaCl 5, KCl 10, MgCl<sub>2</sub> 1, CaCl<sub>2</sub> 1, ethyleneglycoltetraacetic acid (EGTA) 11, HEPES 10, Na<sub>2</sub>ATP 2, Na<sub>2</sub>GTP 0.2 mM. The pH of the intracellular solution was adjusted to 7.4 using CsOH. Various concentrations of AMPA, NMDA, glycine, L-Phe, D-Phe, L-leucine, dizocilpine (MK-801; (+)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,b)cyclohepten-5,10-imine), strychnine, bicuculline and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) were added to the extracellular solution

according to the protocols described below. All compounds were purchased from Sigma Chemical Co., St Louis, MO.

[0080] Statistical data analysis. All measurements are reported as mean  $\pm$  SEM. Statistical analyses were carried out using SigmaStat version 2.03 (SPSS, Inc., Chicago, IL). Before parametric testing, the assumption of normality was validated using the Kolmogorov-Smirnov test with Lilliefors' correction. One way, repeated measures ANOVA followed by Student-Newman-Keuls testing was used to analyze multiple comparisons among control and interventions. When appropriate, paired t testing (two-tailed) was used for single comparisons. A value less than .05 was considered to be significant. All statistics were carried out using raw, untransformed data.

[0081] Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1—The effect of aromatic amino acids (AAAs) on miniature excitatory postsynaptic currents (mEPSCs) in hippocampal neurons

[0082] Hippocampi were dissected from newborn rats and treated with trypsin to dissociate the cells. The hippocampal cells were then resuspended in Neurobasal Medium containing B-27 serum-free supplement, and cultured. Various concentrations of L-Phe, L-Tyr, L-Trp, dizocilpine (MK-801; (+)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,b)cyclohepten-5,10-imine) were added to the extracellular solution. Electrophysiological recordings of miniature excitatory postsynaptic currents (mEPSCs) in cultured rat hippocampal neurons were made in the whole cell configuration. The current data was then digitized and analyzed.

[0083] Figures 1A and 1B show the effect of L-tyrosine, L-phenylalanine, and L-tryptophan on mEPSCs in rat cultured hippocampal neurons. Examples of mEPSCs, recorded in the presence of the NMDA channel blocker MK-801 (10 M) before, during, and after application of L-tyrosine (L-Tyr), L-phenylalanine (L-Phe), or L-Tryptophan (L-Trp) (1000 M) are shown in Figure 1A. Figure 1B shows summary data for 5-8 experiments demonstrating the effect of L-Tyr, L-Trp and L-Phe on the frequency of mEPSCs. The frequencies of events were normalized to control values and plotted against the concentration

of L-Tyr, L-Trp and L-Phe. The results are presented as mean $\pm$ S.E.M. \*,  $P < 0.01$  compared to control. The concentration of glycine was 1 M, whereas the membrane potential was  $-60$  mV.

[0084] Figures 10A-10C show the effect of L-Phe on mEPSCs in rat cultured hippocampal neurons during energy deprivation (ED). To induce ED, glucose was replaced with 2 mM 2-deoxyglucose, and 5 mM sodium cyanide (NaCN) was added. Examples of mEPSCs, recorded in the presence of the NMDA channel blocker MK-801 (10  $\mu$ M) in control, during ED, during ED in the presence of L-Phe (1000  $\mu$ M), and during ED after washout of L-Phe are shown in Figure 10A. Figure 10B shows an example of averaged mEPSCs ( $n = 973$  events in presence of L-Phe;  $n = 3331$  events under control conditions) recorded from the same neuron shown in Figure 10A. Figure 10C shows summary data for four experiments demonstrating the effect of L-Phe on the frequency of mEPSCs. The frequency of mEPSCs during ED was taken as 100%.

[0085] Figures 2A and 2B show the effect of D-tyrosine, D-tryptophan, and D-phenylalanine on the frequency of mEPSCs in rat cultured hippocampal neurons. Examples of mEPSCs, recorded in the presence of the NMDA channel blocker MK-801 before, during and after application of D-phenylalanine (D-Phe), D-tryptophan (D-Trp), and D-tyrosine (D-Tyr) are shown in Figure 2A. Figure 2B shows summary data for 5-8 experiments demonstrating the effect of D-Tyr, D-Trp, and D-Phe on the frequency of mEPSCs. The frequencies of mEPSCs were normalized to control values and plotted against the concentration of D-Tyr, D-Trp, and D-Phe. The results are presented as mean $\pm$ S.E.M. \*,  $P < 0.01$  compared to control.

#### Example 2—Evaluation of the neuroprotective effects of AAAs in hippocampal cell cultures

[0086] Neurons will be subjected to oxygen glucose deprivation (OGD) at 14-16 days in vitro. Neurobasal medium (Gibco/Life Technologies, CA) will be removed and put aside. Glucose-free medium, warmed to 37°C, will be applied to the neurons. Cultures will then be placed into an airtight chamber (Billups-Rothenberg Inc., Del Mar, CA) flushed with 95% N<sub>2</sub>/5% CO<sub>2</sub> until oxygen concentration fell to less than 1%. The chamber should be maintained at 37°C for 1-2.5 hr, the original conditioned Neurobasal medium will be reapplied, with or without AAAs (1mM) and will be returned to the incubator for the duration of the experiment.



[0087] Cell viability at different experimental conditions can be assessed by counting phase-bright cells (live cells) under phase-contrast microscopy and propidium iodide (PI)-labeled nuclei (dead cells) under fluorescence microscopy). Viability can be calculated as the ratio of phase-bright cells to total cells (*i.e.*, phase-bright plus PI-stained) (R. Tremblay *et al.*, 2000). The degree of the leakage of the enzyme lactate dehydrogenase (LGH) will be used as criteria of the necrotic cell damages. The apoptotic-like features can be evaluated by using an enzyme-linked immunosorbent assay (ELISA) with anti-histone/DNA monoclonal antibodies as evidence for DNA damage, and by measuring the caspase activity in rat hippocampal cultures.

Example 3—Middle cerebral artery occlusion (MCAO, Focal ischemia model)

[0088] The rats (Charles Rivers) were anesthetized and have a small skin incision on the neck. Right common carotid artery (CCA), intracarotid artery (ICA) and external carotid artery (ECA) were exposed, then ECA ligated and cut. An occluder made by 4-0 nylon monofilament suture (Ethicon) was introduced through the ECA into ICA, advanced approximately 17 mm intracranially from the CCA bifurcation to make MCAO. Two hours after MCAO procedure, the occluder was withdrawn for the reperfusion under anesthesia. During the operations, the respiratory stability and the pain reaction to the paw pinch were monitored every 5-15 minutes. After the operation the rat was observed to recover from anesthesia checking the respiratory condition and the spontaneous movement. Meanwhile the rats were treated with one of the drugs, L-phenylalanine (L-Phe), 3,5 diiodo-L-tyrosine (DIT), or placebo. The protocol of drug administration consisted of initial single intraperitoneal (ip) injection, 30 min prior to MCAO, followed by 4 ip injections, each every 2 hours for 6 hours since starting reperfusion. Each injection contained 1 ml of 0.9% NaCl (placebo) or 12 mM of DIT. Then the rat was returned to the cage. Three days after the MCAO operation, the rats were deeply anesthetized and a needle was inserted into the apex of the heart, 50 ml of saline and 10U/ml of heparin was infused, followed by decapitation to sample the tissue. The brain was removed, cut to 2 mm thickness and stained with triphenyltetrazolium chloride (TTC) for stroke volume evaluation. To reduce errors associated with processing of tissue for histological analysis, the ischemic volume is presented as the percentage of infarct volume of the contralateral hemisphere (indirect volume calculation).

[0089] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

We claim:

1. A method for treating a neurological condition characterized by excessive activation of glutamatergic ionotropic receptors comprising parenterally administering at least one aromatic amino acid, isomer, or analog thereof, to a patient in need of such treatment.
2. A method for treating a neurological condition characterized by excessive activation of glutamatergic ionotropic receptors comprising parenterally administering at least one aromatic amino acid, isomer, or analog thereof, to a patient in need of such treatment, wherein the neurological condition is not Parkinson's disease.
3. The method, according to claim 2, wherein the neurological condition is selected from the group consisting of anoxic damage, hypoxic damage, traumatic brain injury, spinal cord injury, local anesthetic-induced seizure activity, ischemic stroke, ischemic neurodegeneration of the retina, epilepticus, Tourette's syndrome, obsessive-compulsive disorder, drug-induced CNS injury, chronic pain syndromes, lateral sclerosis, Alzheimer's disease, Huntington's chorea, AIDS dementia syndrome, and cocaine addiction, or combinations thereof.
4. The method, according to claim 2, wherein the neurological condition is not a condition selected from the group consisting of multi-infarct dementia, Alzheimer's disease, and cocaine addiction.
5. The method, according to claim 2, wherein the neurological condition is selected from the group consisting of ischemia, stroke, traumatic brain injury, hypoglycemia, and epileptic seizures.
6. The method, according to claim 2, wherein the isomer is not D-phenylalanine.
7. The method, according to claim 2, wherein the patient is suffering from the neurological condition.
8. The method, according to claim 2, wherein the aromatic amino acid, isomer, or analog thereof, is administered to the patient intravenously.

9. The method, according to claim 2, wherein the aromatic amino acid, isomer, or analog thereof, is administered to the patient intra-nasally.

10. The method, according to claim 2, wherein the aromatic amino acid, isomer, or analog thereof, is administered in an amount sufficient to raise the concentration of the aromatic amino acid, isomer, or analog to above a physiologically normal level.

11. The method, according to claim 2, wherein the aromatic amino acid, isomer, or analog thereof, is administered in an amount sufficient to raise the patient's blood plasma level of the aromatic amino acid, isomer, or analog, to within a range of about 200  $\mu$ M to about 2000  $\mu$ M.

12. The method, according to claim 2, wherein the aromatic amino acid, isomer, or analog thereof, is administered in an amount sufficient to raise the patient's blood plasma level of the aromatic amino acid, isomer, or analog, to within a range of about 300  $\mu$ M to about 1800  $\mu$ M.

13. The method, according to claim 2, wherein the aromatic amino acid, isomer, or analog thereof, is administered in an amount sufficient to raise the patient's blood plasma level of the aromatic amino acid, isomer, or analog, to within a range of about 800  $\mu$ M to about 1500  $\mu$ M.

14. The method, according to claim 2, wherein said aromatic amino acid is selected from the group consisting of L-tyrosine, L-tryptophan, and L-phenylalanine.

15. The method, according to claim 2, wherein a mixture of said aromatic amino acids are administered, and wherein said mixture is selected from the group consisting of: L-tyrosine and L-tryptophan; L-tyrosine and L-phenylalanine; L-tryptophan and L-phenylalanine; and L-tyrosine, L-tryptophan, and L-phenylalanine.

16. The method, according to claim 2, wherein said aromatic amino acid isomer is an enantiomer selected from the group consisting of D-tyrosine, D-tryptophan, and D-phenylalanine.

17. The method, according to claim 2, wherein a mixture of said aromatic amino acid isomers are administered, and wherein said mixture is selected from the group consisting of:

D-tyrosine and D-tryptophan; D-tyrosine and D-phenylalanine; D-tryptophan and D-phenylalanine; and D-tyrosine, D-tryptophan, and D-phenylalanine.

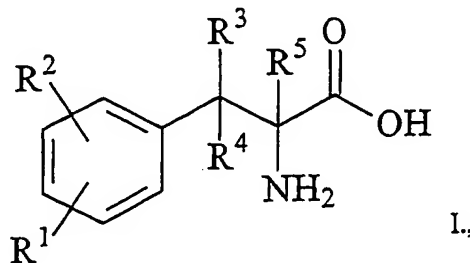
18. The method, according to claim 2, wherein a mixture of said aromatic amino acid and said isomer is administered, wherein said mixture comprises a levorotatory aromatic amino acid and a dextrorotatory aromatic amino acid.

19. The method, according to claim 2, wherein in a mixture of said aromatic amino acid and said isomer is administered, and said mixture comprises L-phenylalanine and D-phenylalanine.

20. The method, according to claim 2, wherein said aromatic amino acid, isomer, or analog is co-administered with a facilitating substance that increases transport of said aromatic amino acid, isomer, or analog across the blood-brain barrier.

21. The method, according to claim 20, wherein said facilitating substance is an allosteric enhancer.

22. The method, according to claim 2, wherein the analog has the structure of formula I

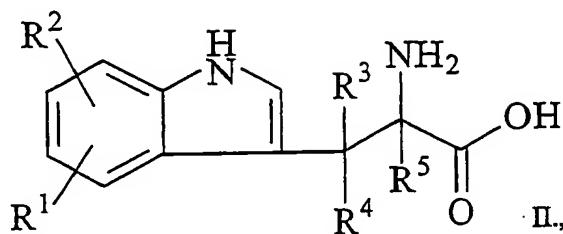


wherein  $R^1$  and  $R^2$ , which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^3$  is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^4$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein  $R^4$  is not present when  $R^3$  is O; and wherein  $R^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

23. The method, according to claim 22, wherein  $R^3$  and  $R^4$  together form a ring structure selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl.

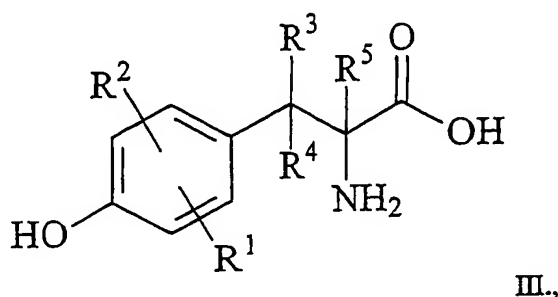
24. The method, according to claim 23, wherein the ring structure is optionally benzofused at any available position.

25. The method, according to claim 2, wherein the analog has the structure of formula II



wherein  $R^1$  and  $R^2$ , which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^3$  is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^4$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein  $R^4$  is not present when  $R^3$  is O; and wherein  $R^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

26. The method, according to claim 2, wherein the analog has the structure of formula III



wherein  $R^1$  and  $R^2$ , which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^3$  is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^4$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein  $R^4$  is not present when  $R^3$  is O; and wherein  $R^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

27. The method, according to claim 26, wherein  $R^3$  and  $R^4$  together form a ring structure selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl.

28. The method, according to claim 27, wherein the ring structure is optionally benzofused at any available position.

29. The method, according to claim 2, wherein the analog is 3,5-diiodo-L-tyrosine or 3,5-dibromo-L-tyrosine.

30. A method for modulating the activity of a glutamate receptor comprising contacting an aromatic amino acid, isomer, or analog thereof, with said receptor, wherein said aromatic amino acid, isomer, or analog thereof, modulates the activity of said receptor.

31. The method, according to claim 30, wherein said aromatic amino acid, isomer, or analog thereof, stimulates the activity of said glutamate receptor.

32. The method, according to claim 30, wherein said aromatic amino acid, isomer, or analog thereof, inhibits the activity of said glutamate receptor.

33. The method, according to claim 30, wherein said glutamate receptor is an ionotropic glutamate receptor.

34. The method, according to claim 30, wherein said glutamate receptor is an metabotropic glutamate receptor.

35. The method, according to claim 30, wherein said aromatic amino acid, isomer, or analog thereof is contacted with said receptor *in vitro*.

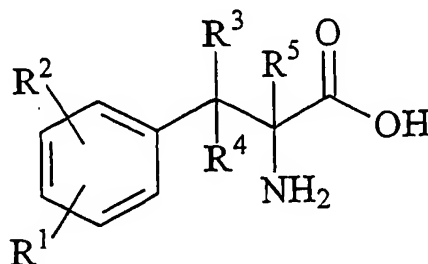
36. The method, according to claim 30, wherein said aromatic amino acid, isomer, or analog thereof is contacted with said receptor *in vivo*.

37. The method, according to claim 30, wherein said aromatic amino acid is selected from the group consisting of L-tyrosine, L-tryptophan, and L-phenylalanine.

38. The method, according to claim 30, wherein a mixture of said aromatic amino acid and said isomer is contacted with said receptor, and wherein said mixture comprises a levorotatory aromatic amino acid and a dextrorotatory aromatic amino acid.

39. The method, according to claim 30, wherein the isomer is not D-phenylalanine.

40. The method, according to claim 30, wherein the analog has the structure of formula I



I.,

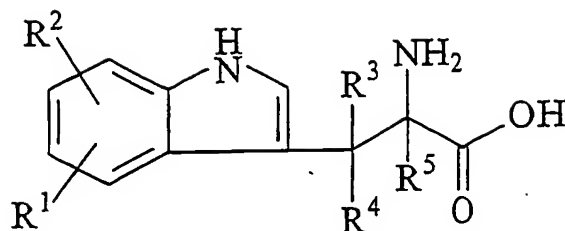
wherein R<sup>1</sup> and R<sup>2</sup>, which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein R<sup>3</sup> is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein R<sup>4</sup> is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein R<sup>4</sup> is not present when R<sup>3</sup> is O; and wherein R<sup>5</sup> is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

41. The method, according to claim 40, wherein R<sup>3</sup> and R<sup>4</sup> together form a ring structure selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl.

42. The method, according to claim 41, wherein the ring structure is optionally benzofused at any available position.

43. The method, according to claim 30, wherein the analog has the structure of formula II

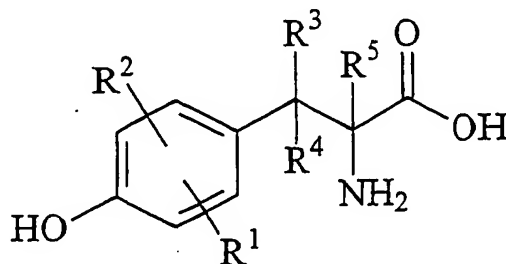




II.,

wherein  $R^1$  and  $R^2$ , which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^3$  is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^4$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein  $R^4$  is not present when  $R^3$  is O; and wherein  $R^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

44. The method, according to claim 30, wherein the analog has the structure of formula III



III.,

wherein  $R^1$  and  $R^2$ , which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^3$  is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^4$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein  $R^4$  is not present when  $R^3$  is O; and wherein  $R^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

45. The method, according to claim 44, wherein  $R^3$  and  $R^4$  together form a ring structure selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl.

46. The method, according to claim 45, wherein the ring structure is optionally benzofused at any available position.

47. The method, according to claim 30, wherein the analog is 3,5-diiodo-L-tyrosine or 3,5-dibromo-L-tyrosine.

48. A pharmaceutical composition useful in treating a neurological condition characterized by overactivation of an ionotropic glutamatergic receptor, said composition comprising an aromatic amino acid, isomer, or analog thereof, and a pharmaceutically acceptable carrier or diluent.

49. The pharmaceutical composition of claim 48, wherein said pharmaceutical composition further comprises a facilitating substance that increases transport of said aromatic amino acid, isomer, or analog, across the blood-brain barrier.

50. The pharmaceutical composition of claim 47, wherein said facilitating substance is an allosteric enhancer.

51. The pharmaceutical composition of claim 48, wherein said aromatic amino acid is selected from the group consisting of L-tyrosine, L-tryptophan, and L-phenylalanine.

52. The pharmaceutical composition of claim 48, wherein said pharmaceutical composition comprises a mixture of said aromatic amino acids selected from the group consisting of: L-tyrosine and L-tryptophan; L-tyrosine and L-phenylalanine; L-tryptophan and L-phenylalanine; and L-tyrosine, L-tryptophan, and L-phenylalanine.

53. The pharmaceutical composition of claim 48, wherein said aromatic amino acid isomer is an enantiomer selected from the group consisting of D-tyrosine, D-tryptophan, and D-phenylalanine.

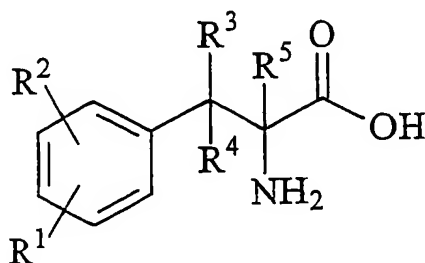
54. The pharmaceutical composition of claim 48, wherein said pharmaceutical composition comprises a mixture of said aromatic amino acid isomers selected from the group consisting of: D-tyrosine and D-tryptophan; D-tyrosine and D-phenylalanine; D-tryptophan and D-phenylalanine; and D-tyrosine, D-tryptophan, and D-phenylalanine.

55. The pharmaceutical composition of claim 48, wherein said pharmaceutical composition comprises a mixture of aromatic amino acids and enantiomers thereof consisting of a dextrorotatory amino acid and a levorotatory amino acid.

56. The pharmaceutical composition of claim 48, wherein said aromatic amino acid is a mixture of L-phenylalanine and D-phenylalanine.

57. The pharmaceutical composition of claim 48, wherein said isomer is not D-phenylalanine.

58. The pharmaceutical composition of claim 48, wherein said analog has the structure of formula I



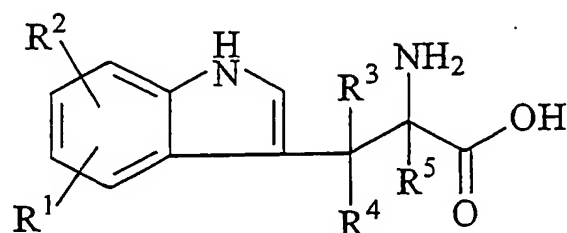
I.,

wherein R<sup>1</sup> and R<sup>2</sup>, which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein R<sup>3</sup> is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein R<sup>4</sup> is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein R<sup>4</sup> is not present when R<sup>3</sup> is O; and wherein R<sup>5</sup> is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

59. The pharmaceutical composition of claim 58, wherein R<sup>3</sup> and R<sup>4</sup> together form a ring structure selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl.

60. The pharmaceutical composition of claim 59, wherein said ring structure is optionally benzofused at any available position.

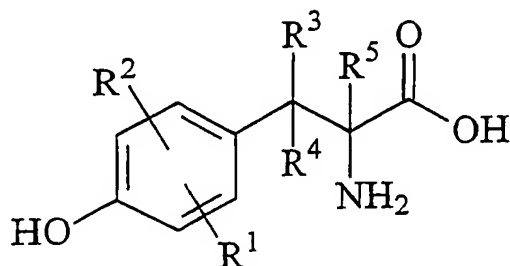
61. The pharmaceutical composition of claim 48, wherein said analog has the structure of formula II



II.,

wherein  $R^1$  and  $R^2$ , which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^3$  is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^4$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but wherein  $R^4$  is not present when  $R^3$  is O; and wherein  $R^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

62. The pharmaceutical composition of claim 48, wherein the analog has the structure of formula III



III.,

wherein  $R^1$  and  $R^2$ , which may be the same or different, are each selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^3$  is selected from the group consisting of H, O, alkyl, alkenyl, alkynyl, halogen, and alkoxy; wherein  $R^4$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy, but

wherein  $R^4$  is not present when  $R^3$  is O; and wherein  $R^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen, and alkoxy.

63. The pharmaceutical composition of claim 62, wherein  $R^3$  and  $R^4$  together form a ring structure selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl.

64. The pharmaceutical composition of claim 63, wherein said ring structure is optionally benzofused at any available position.

65. The pharmaceutical composition of claim 48, wherein said analog is 3,5-diido-L-tyrosine or 3,5-dibromo-L-tyrosine.

66. An article of manufacture useful in treating a neurological condition characterized by overactivation of an ionotropic glutamatergic receptor, said article containing a pharmaceutical composition comprising an aromatic amino acid, isomer, or analog thereof, and a pharmaceutically acceptable carrier or diluent.

67. The article of manufacture of claim 66, wherein said article is an intravenous bag.

68. The article of manufacture of claim 66, wherein said article is selected from the group consisting of a syringe, a nasal applicator, and a microdialysis probe.

69. The article of manufacture of claim 66, wherein said article further comprises printed materials disclosing instructions for the parenteral treatment of the neurological condition.

70. The article of manufacture of claim 66, wherein the printed material is embossed or imprinted on the article of manufacture and indicates the amount or concentration of aromatic amino acid, isomer, or analog thereof, recommended doses for parenteral treatment of the neurological condition, or recommended weights of patients to be treated.

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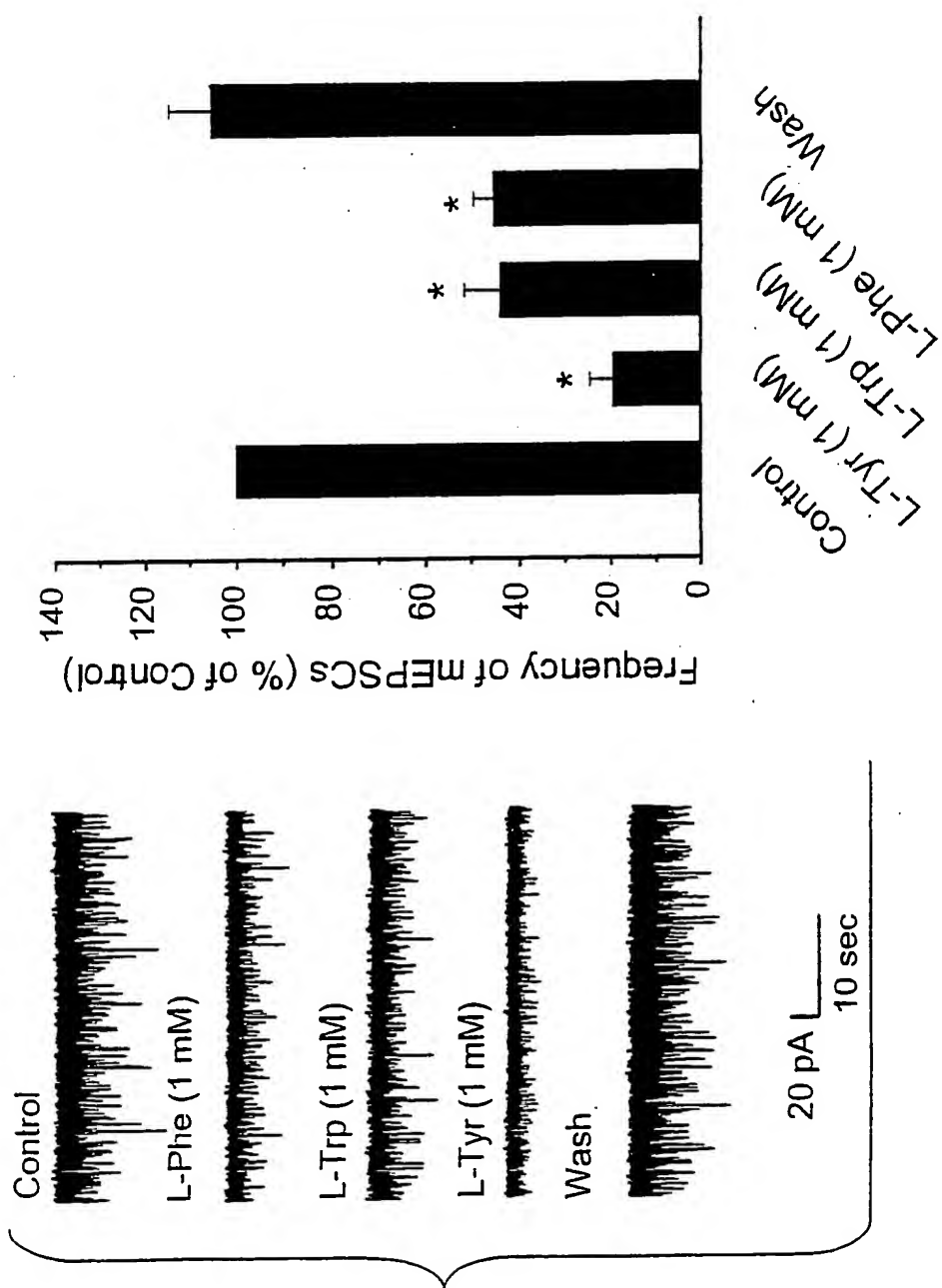


FIG. 1A

FIG. 1B

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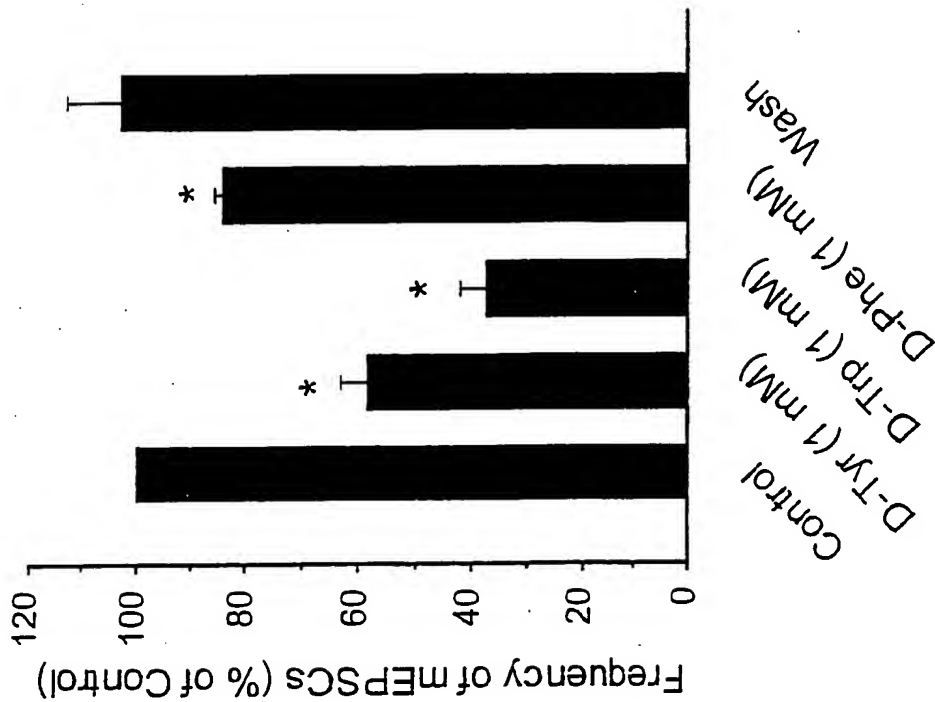


FIG. 2B

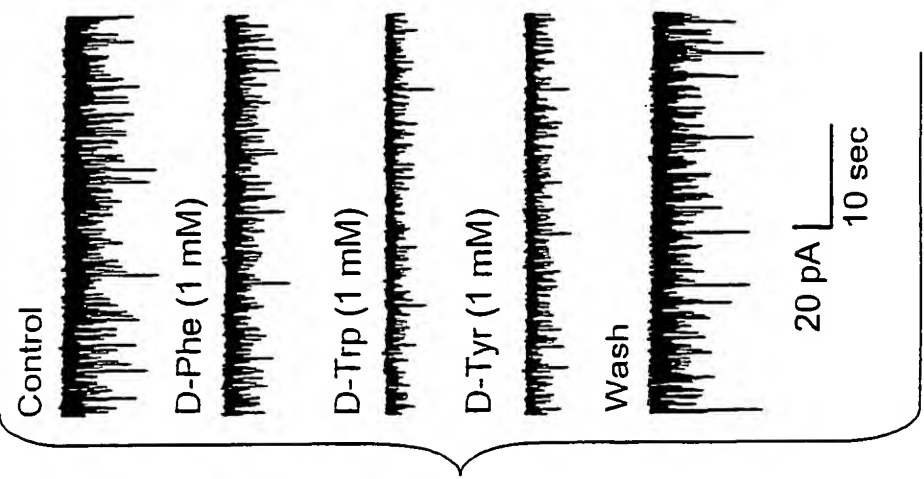


FIG. 2A

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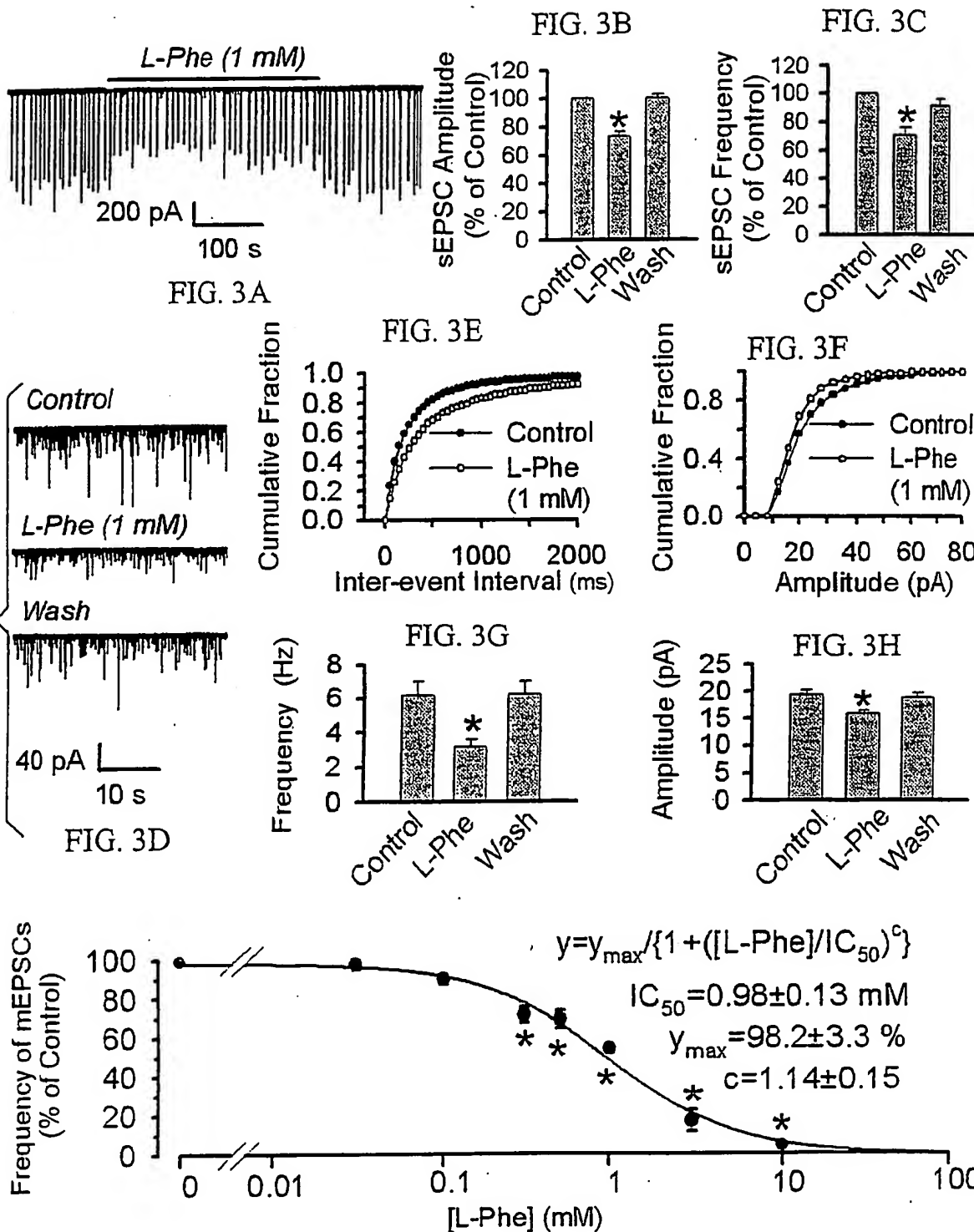


FIG. 3I



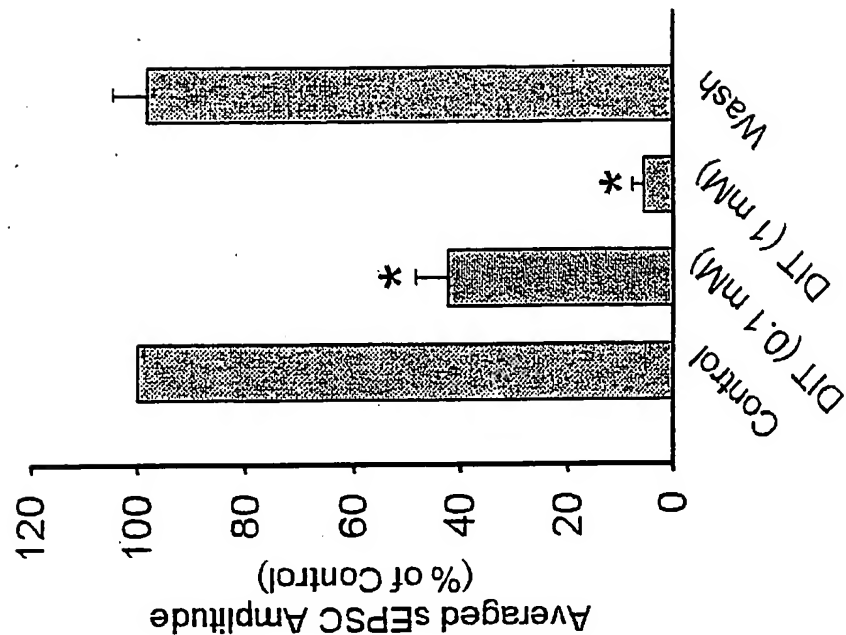


FIG. 4C

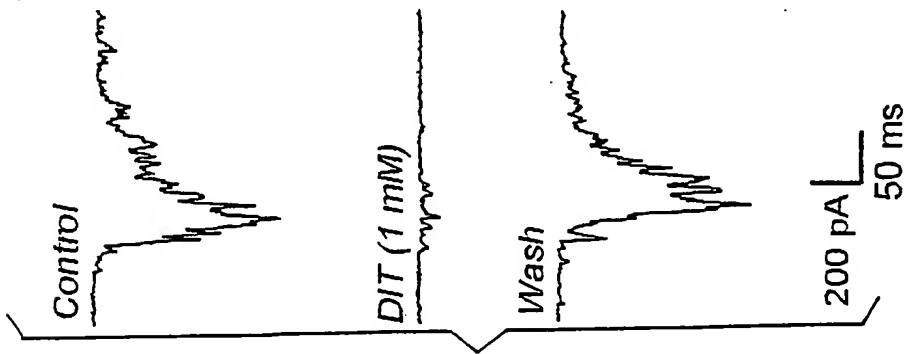


FIG. 4B

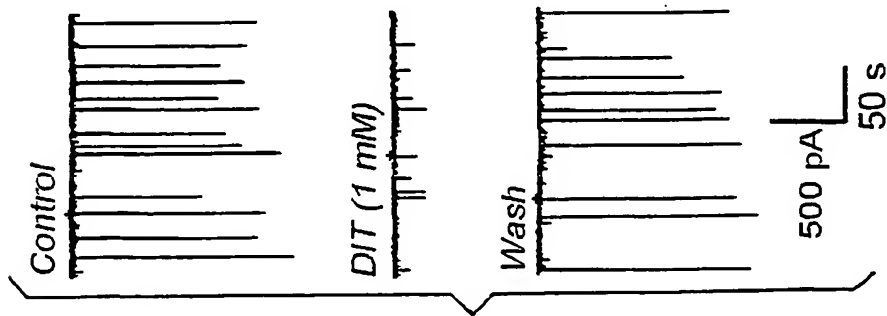


FIG. 4A

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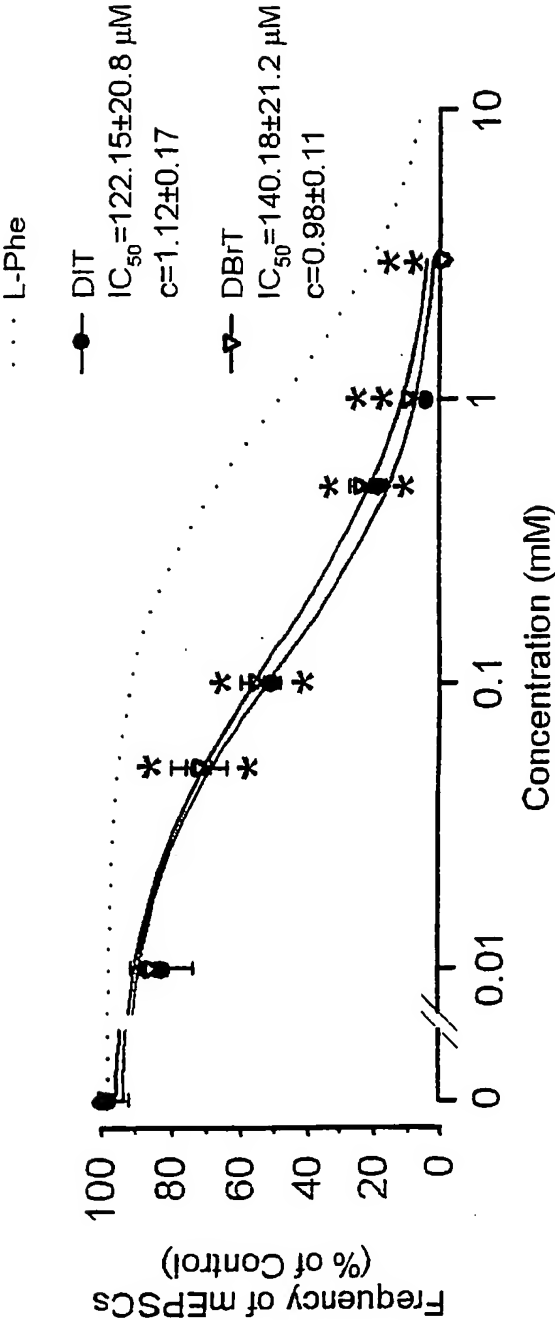


FIG. 4D

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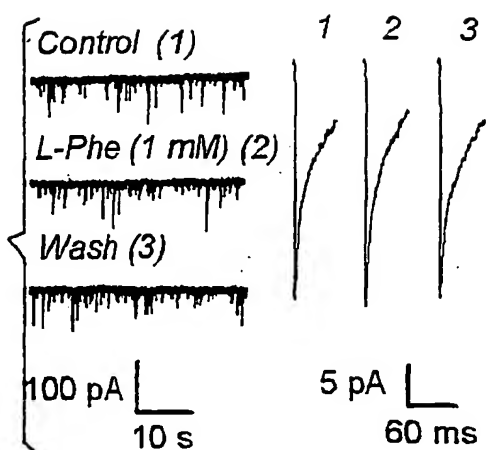


FIG. 5A

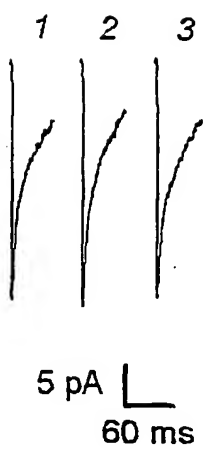


FIG. 5B

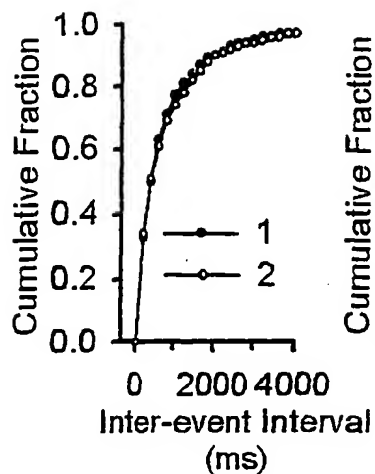


FIG. 5C

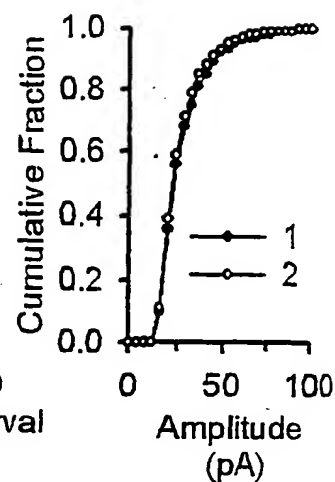


FIG. 5D

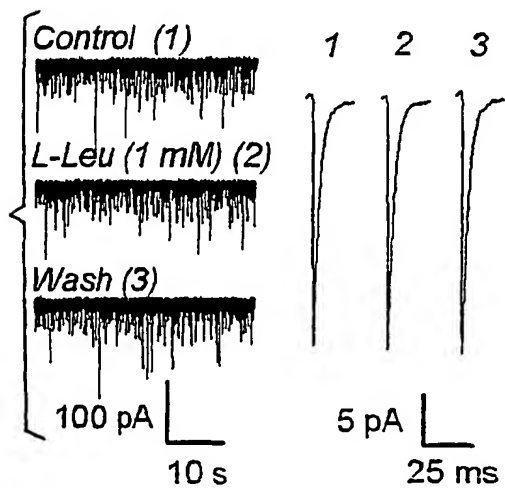


FIG. 5E



FIG. 5F

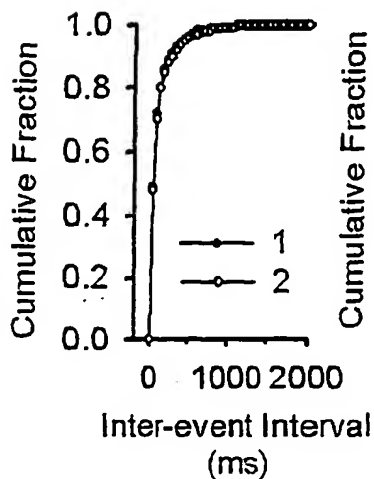


FIG. 5G

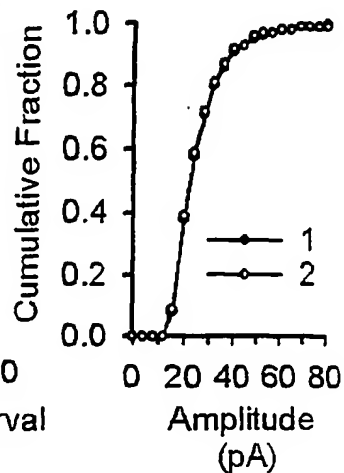


FIG. 5H

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FIG. 6A

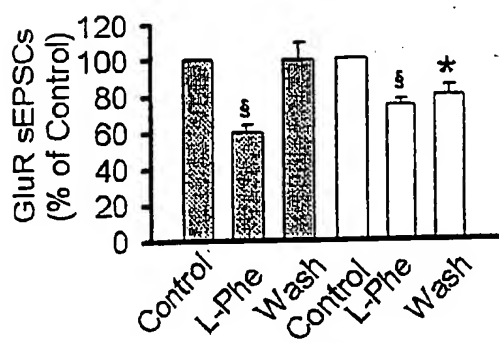
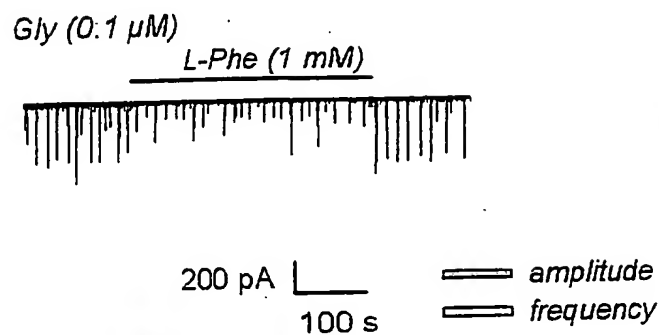


FIG. 6B

FIG. 6C

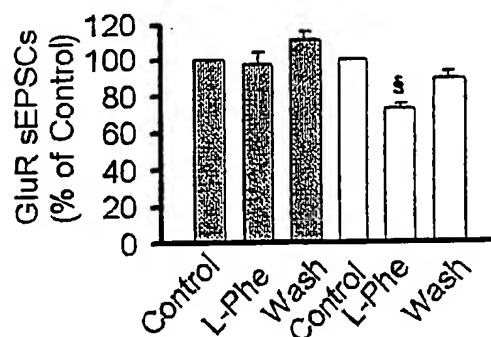
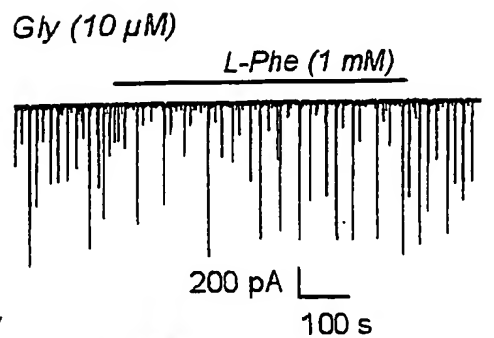


FIG. 6D

FIG. 6E

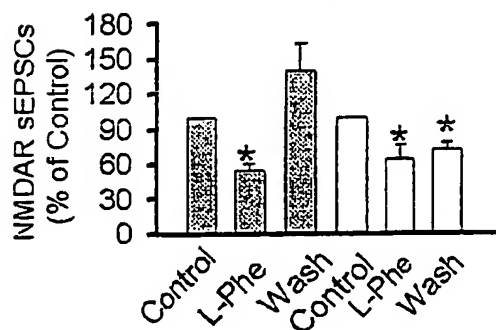
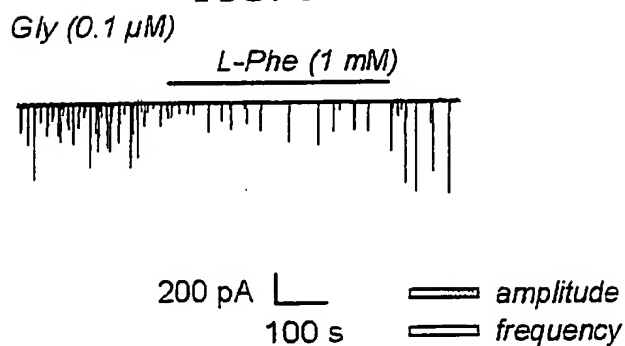


FIG. 6F

FIG. 6G

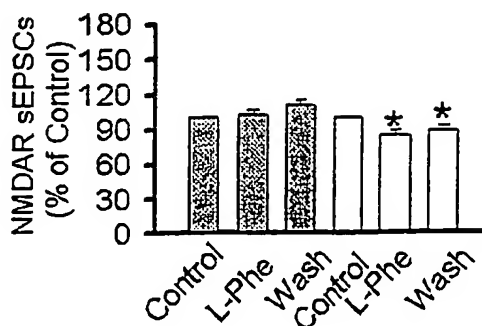
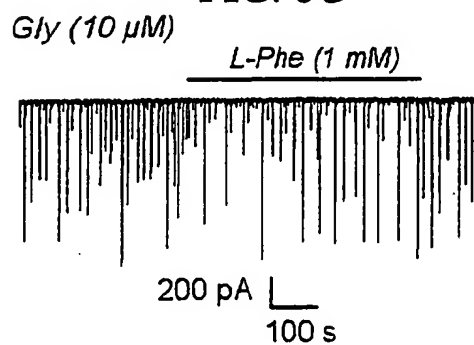


FIG. 6H

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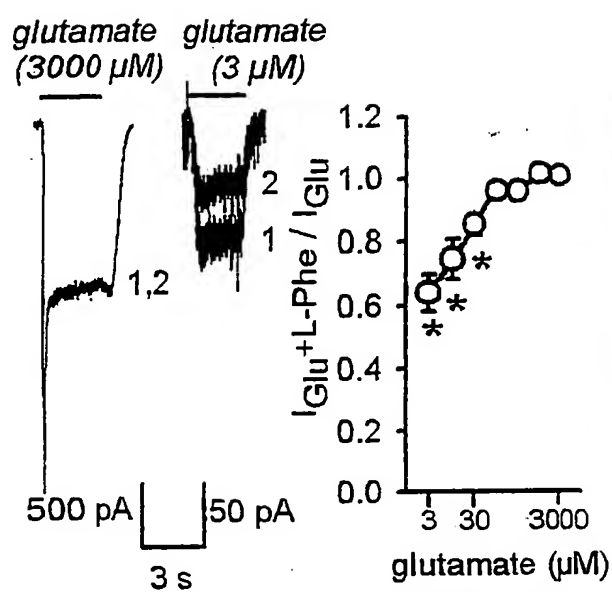


FIG. 7A

FIG. 7B

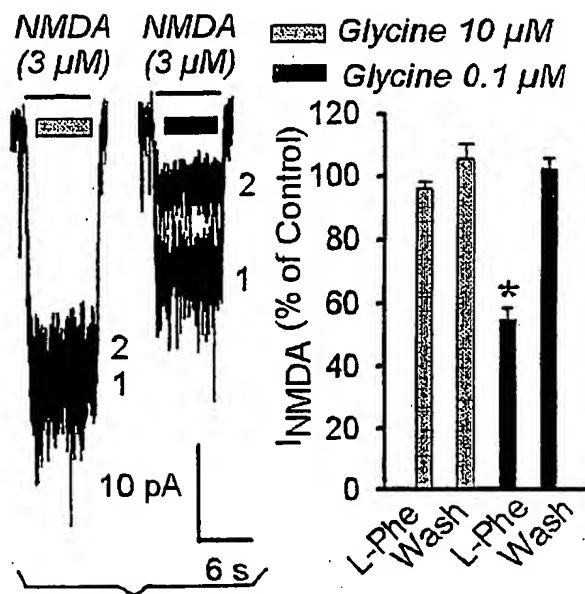


FIG. 7C

FIG. 7D

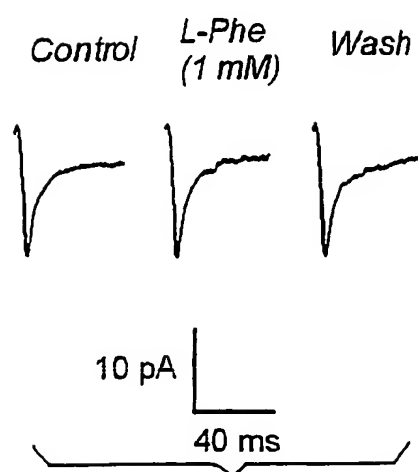


FIG. 7E

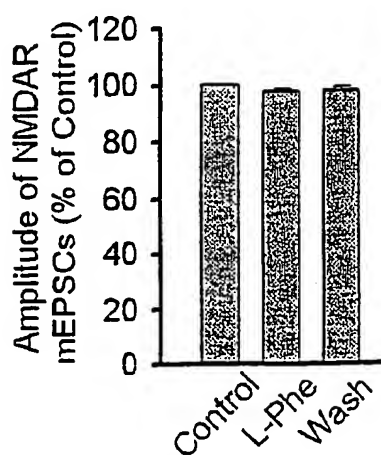


FIG. 7F

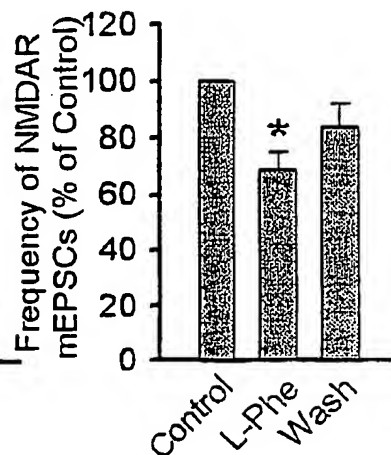


FIG. 7G

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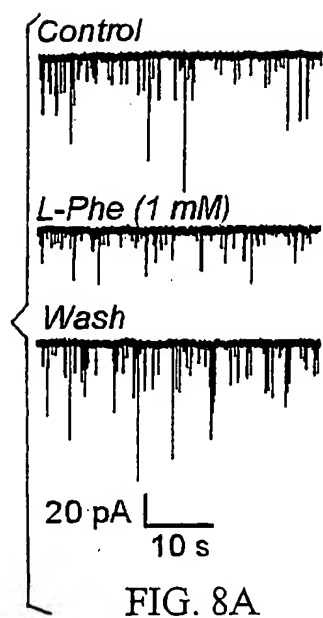


FIG. 8A

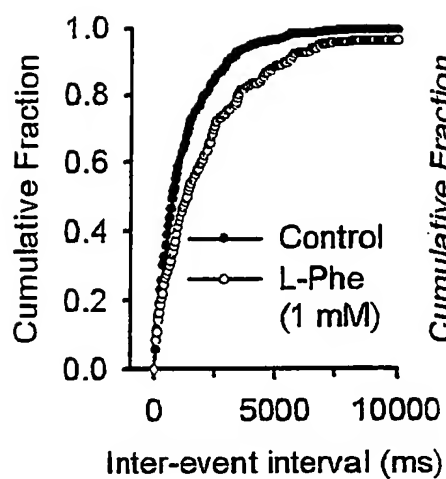


FIG. 8B

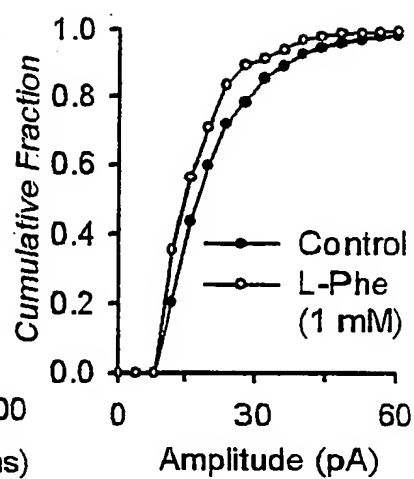


FIG. 8C

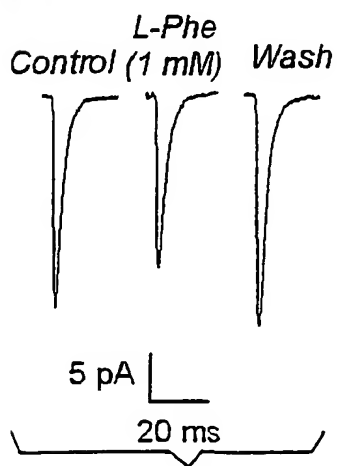


FIG. 8D

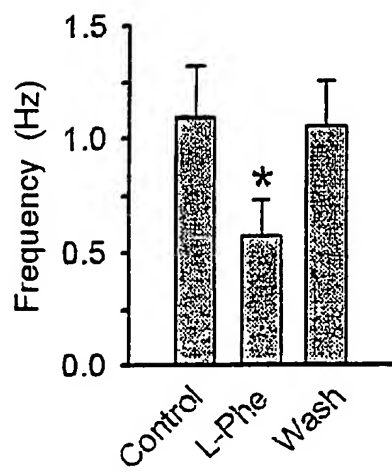


FIG. 8E

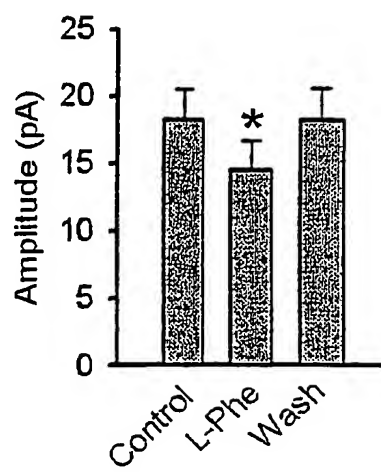


FIG. 8F

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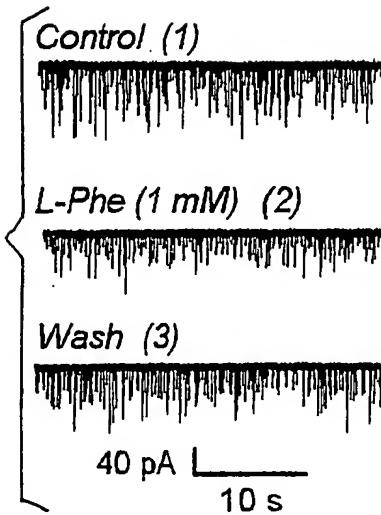


FIG. 9A

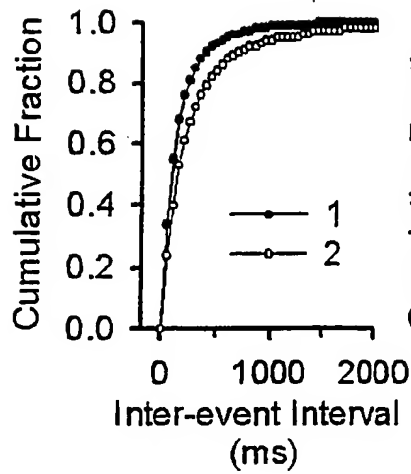


FIG. 9B

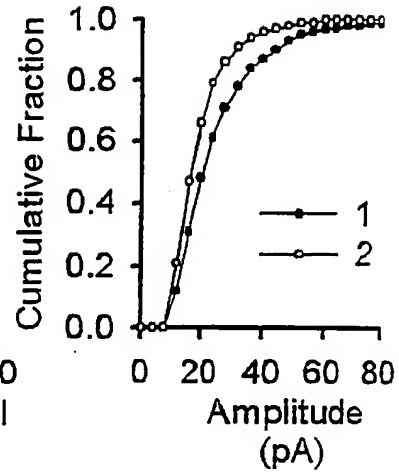


FIG. 9C

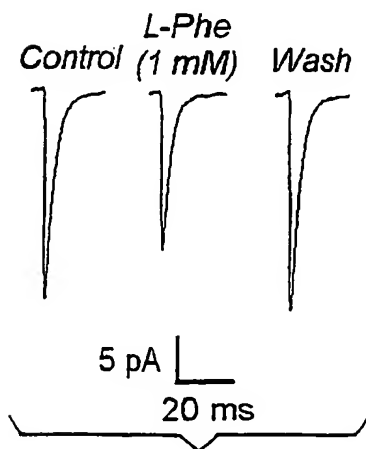


FIG. 9D

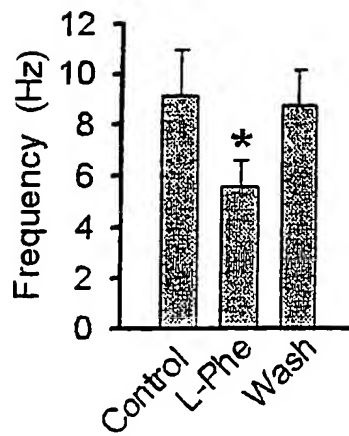


FIG. 9E

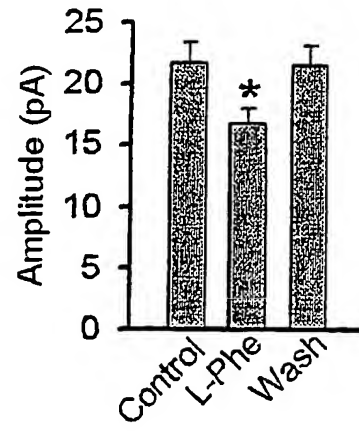


FIG. 9F

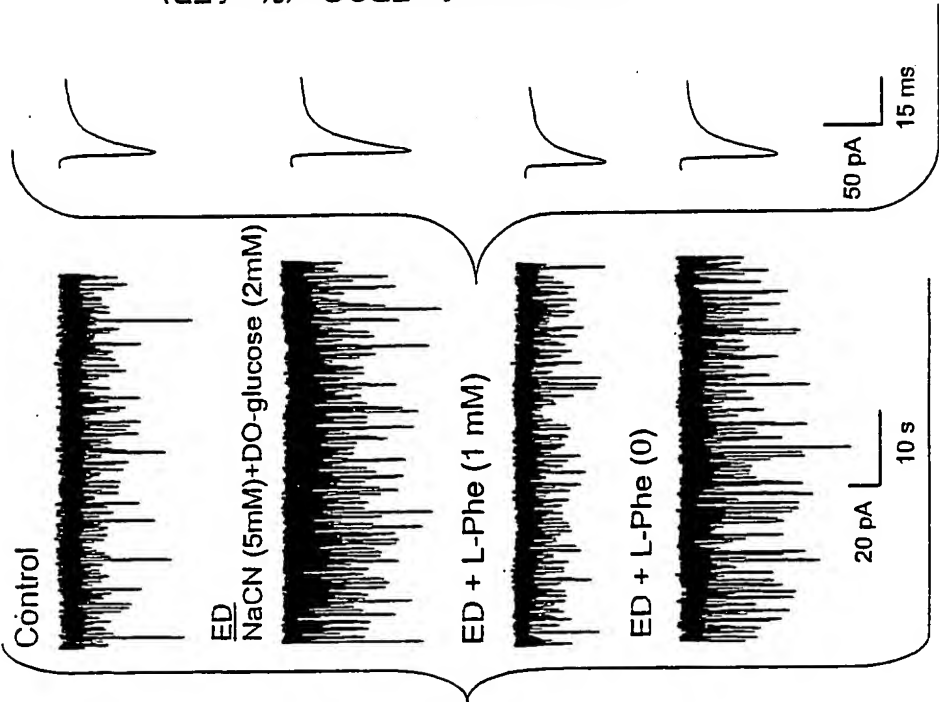


FIG. 10A

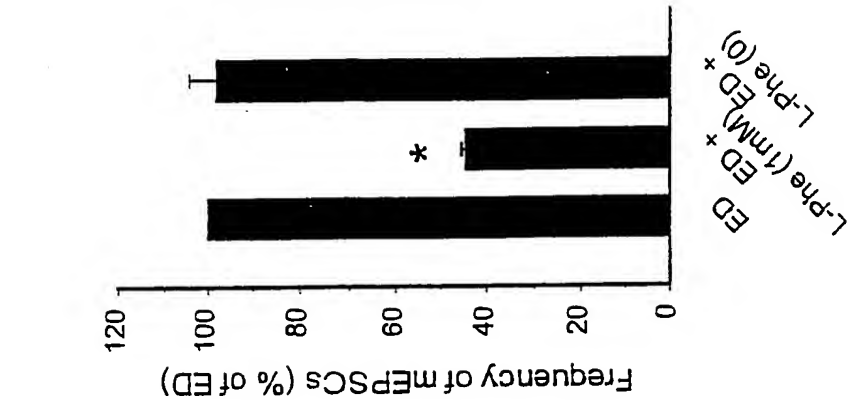


FIG. 10B



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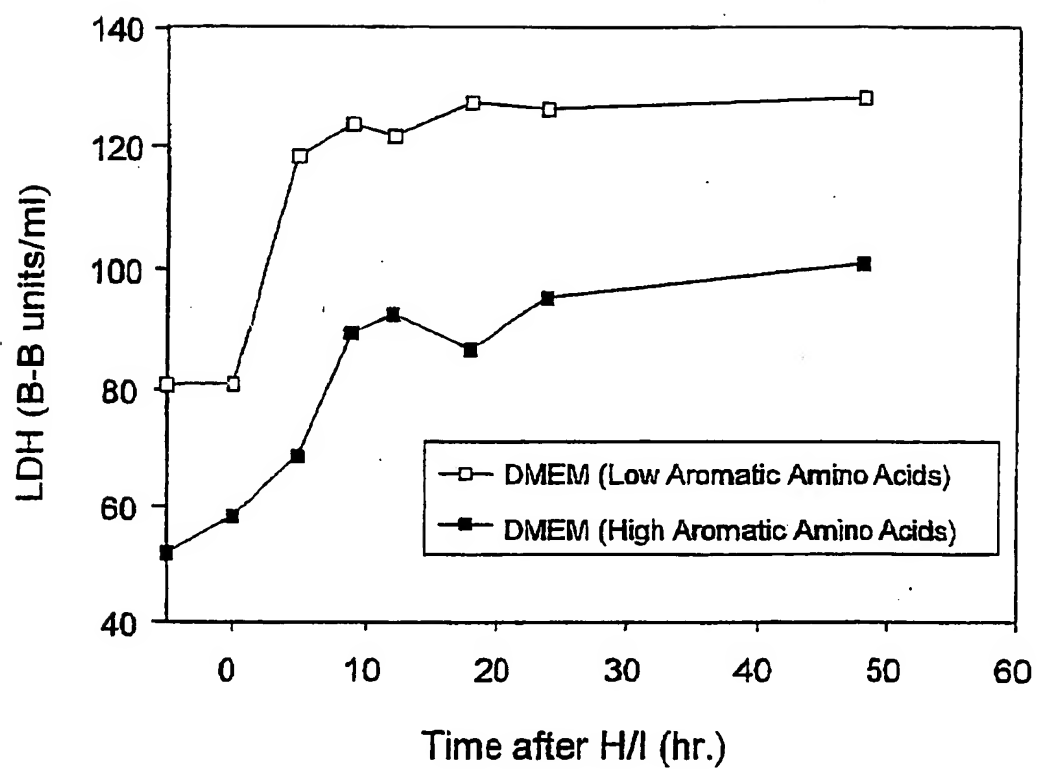


FIG. 11A

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FIG. 11B

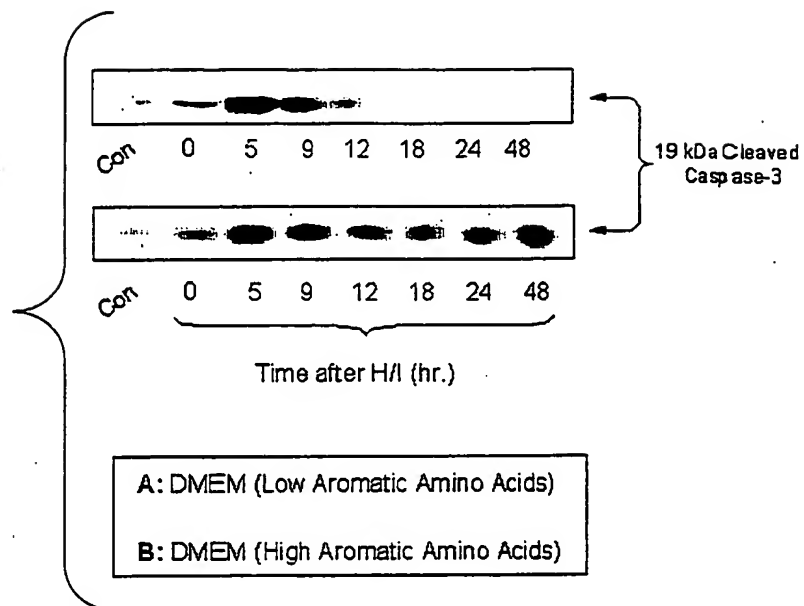


FIG. 12A

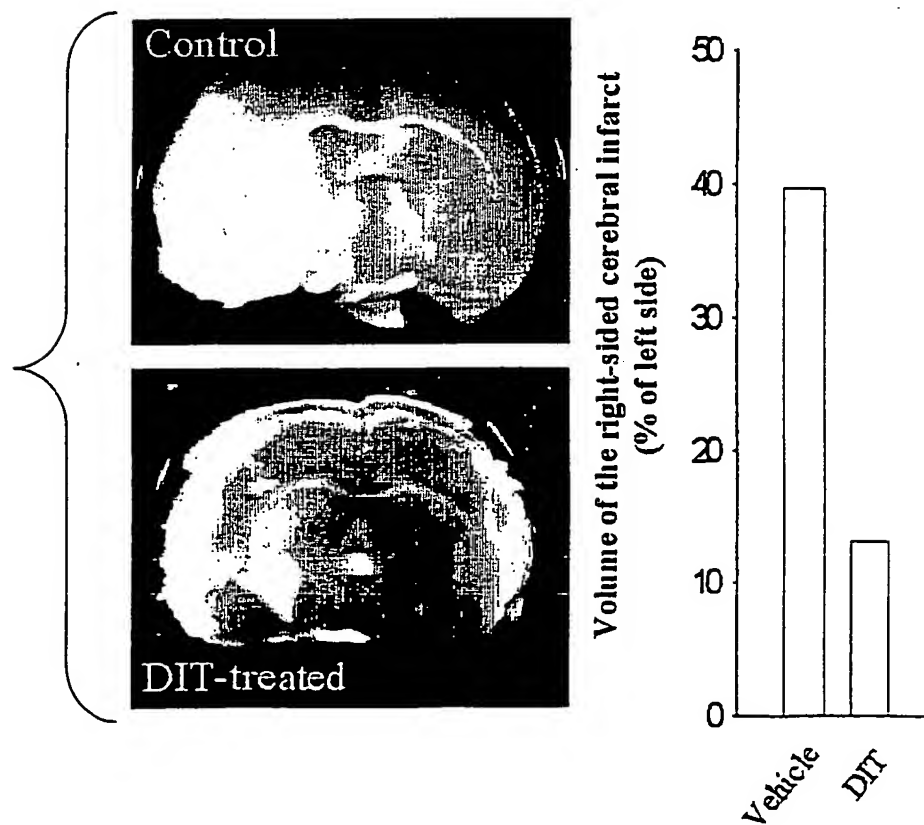


FIG. 12B

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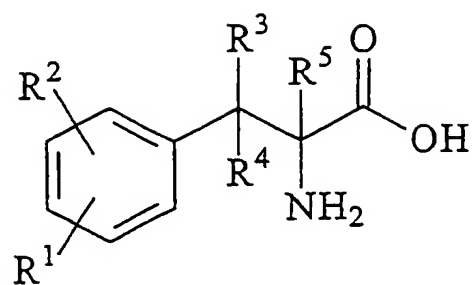


FIG. 13

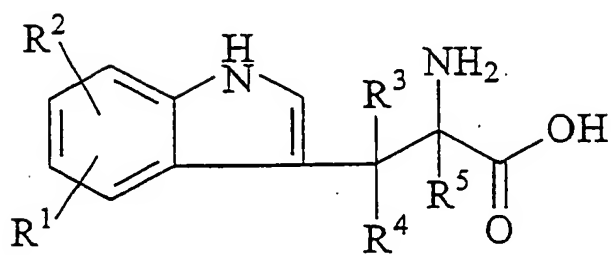


FIG. 14

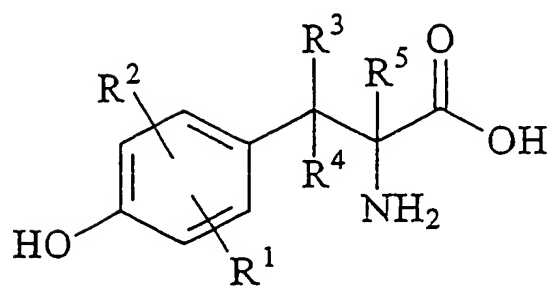


FIG. 15

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